Searching for microbes
Part X.
Reactions with labelled components

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To practical of VLLM0421c

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Content of this slideshow

- Classes of antibodies
- Reactions with labelled components: survey
- Immunofluorescence and RIA
- ELISA: principle
- ELISA: practical reading
- Western blotting

Tale

- There was a sailor, and she had various object on the board bound so to keep them in the ship. **She had her trieder bound to the rescue circle, the rescue circle to rescue boat, and the rescue boat to the board.** So event the highest wawes were not able to take the objects away.
- Once the sailor‘s husband came. He wanted to assay the **rescue circle. He bound the trieder away and he bound the circle from the boat.**
- **A wave came – and the trieder was flown away from the board.**

The original tale about a male sailor and his wife was changed in order to fight with gender stereotypes.

What to learn from the tale

- The principle of reactions of labelled components: We bind one component to another; after each step, the flowing out of components comes.
- **This process takes away everything that is not bound**
- A negative reaction = a reaction, where one component of the chain of components bound one to another is missing. **The other components are not bound to the surface, and they are flown away.**

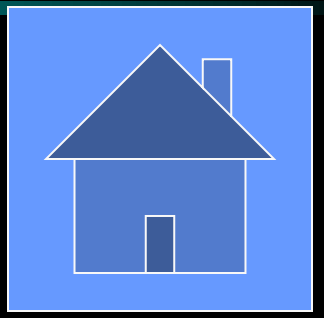
Classes of antibodies

Course of antibody answer

- Class IgM is formed as first, but it is also first to disappear. They do not come through placenta, in a newborn it certifies its own infection
- Class IgG is formed later and remains as memory antibodies. They are able to pass through placenta.

(a newborn may have them from its mother)

Other classes of antibodies



- Class IgA is examined in some infections instead of IgM. This class is important mainly in mucous membrane immunity, so in infections entering the body through mucous membrane (e. g. gastrointestinal)
- Class IgE are present in allergy and helmint infecions. Specific IgE against a certain pathogen are rarelly examined
- Class IgD is not examined im microbiology

Reaction with labelled components: survey

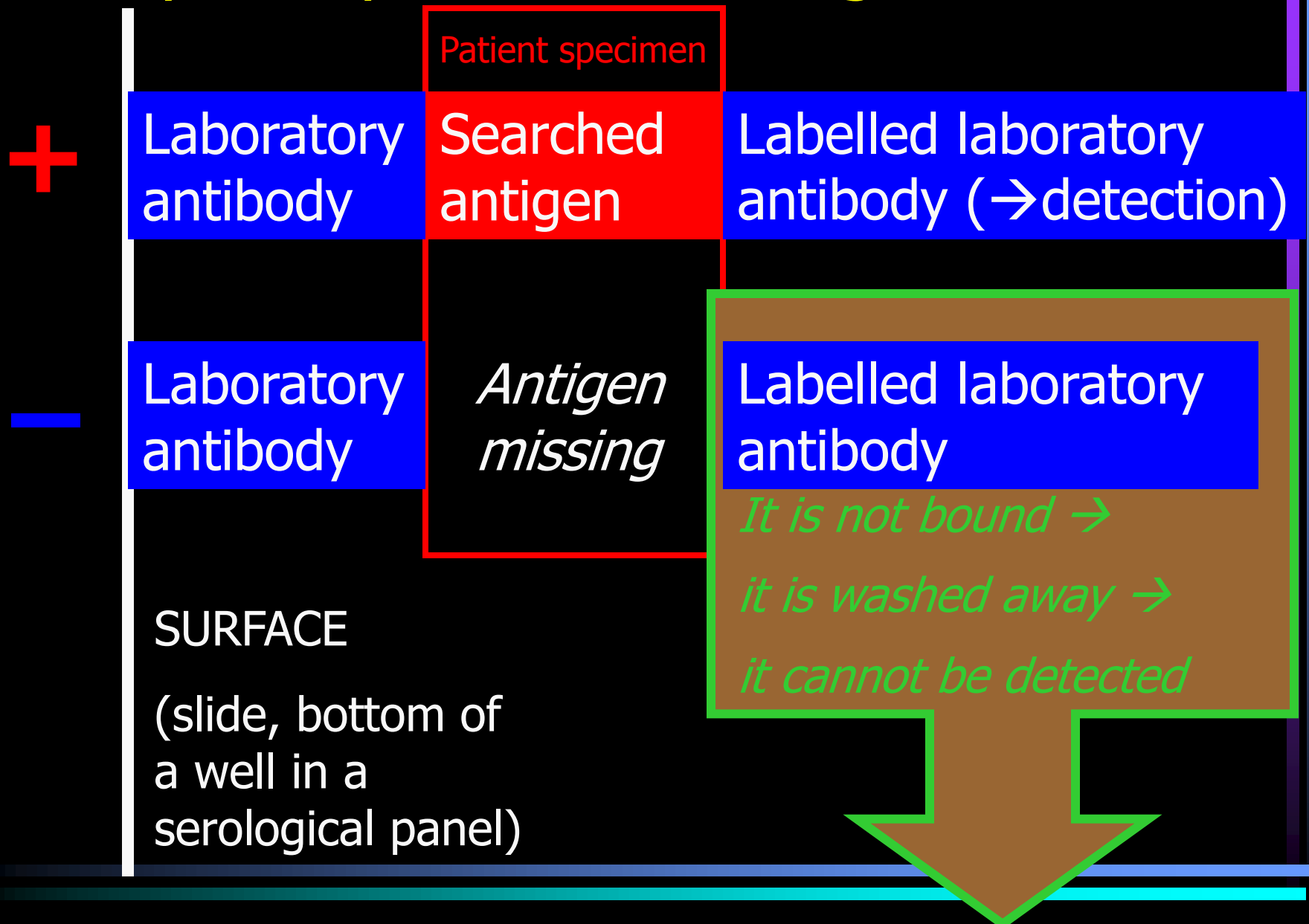
Reactions with labelled components

- Individual components are bound on the previous components, the first of them to the surface.
- Instead of one component a specimen from pacient is used. The specimen is suspicious to contain the given component.
- If it is true, the component is bound
- When all components bind respectivelly, a not-interrupted chain is formed
- At the end there is a labelling agent

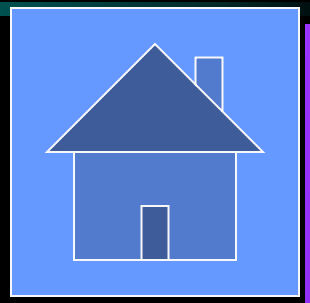
Washing out and its sense

- When also the components that are not bound to the surface would remain, we would not be able to differenciate a positive reaction and a negative one.
- That is why after each step washing follows. After such a washing, only bound components remain present.
- When the chain is broken, the part after the missing component is washed out.

Example of positive and negative course



Types of labelling agent

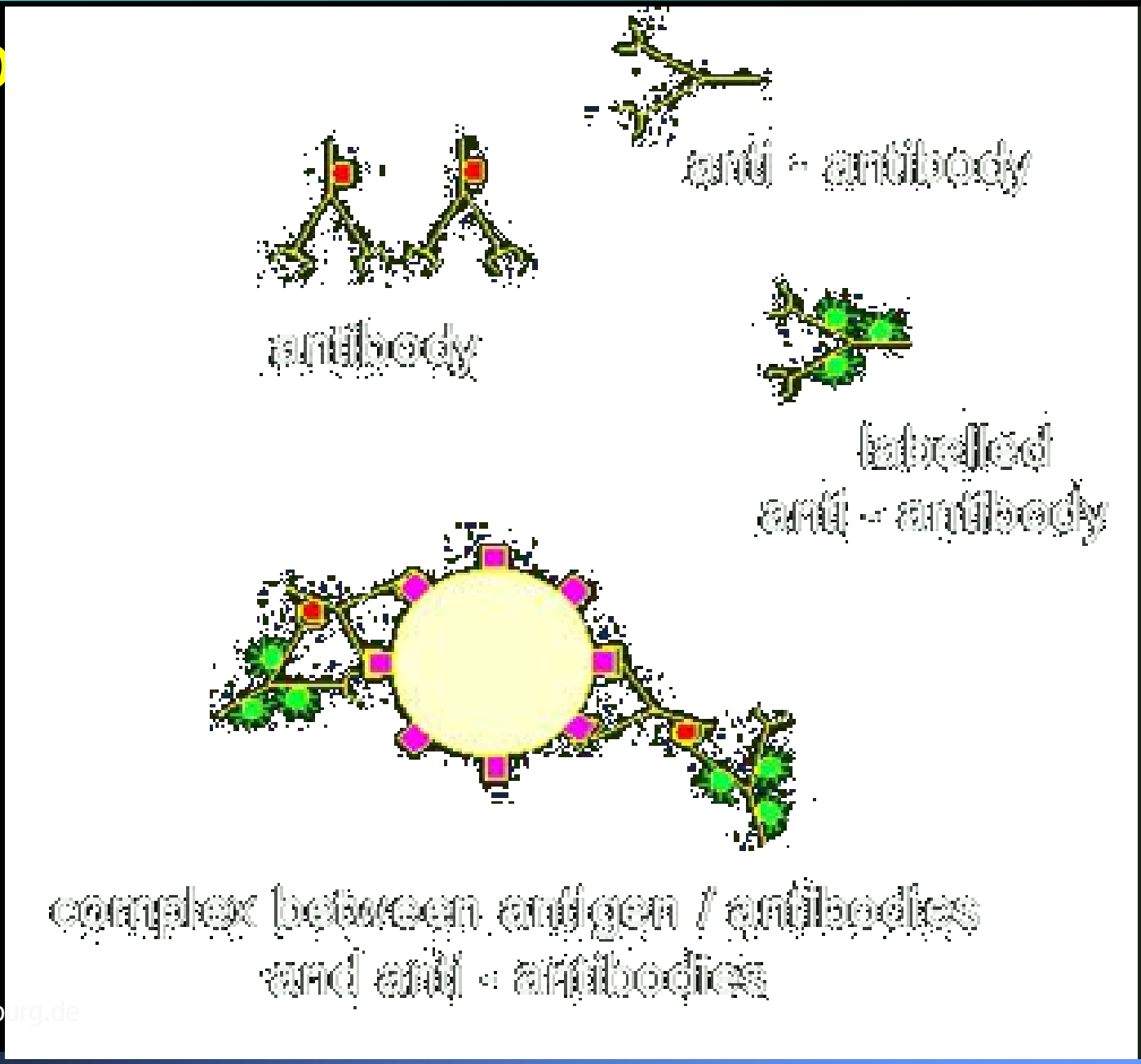


- Fluorescent dye is labelling agent in immunofluorescence
- Radioisotope is labelling agent in RIA
- Enzyme is labelling agent in ELISA
  - Western blotting is a special type of an ELISA, where individual antigens are divided electroforetically

When an enzyme is used as a labelling agent, the very last component should be the substrate – so one more component.

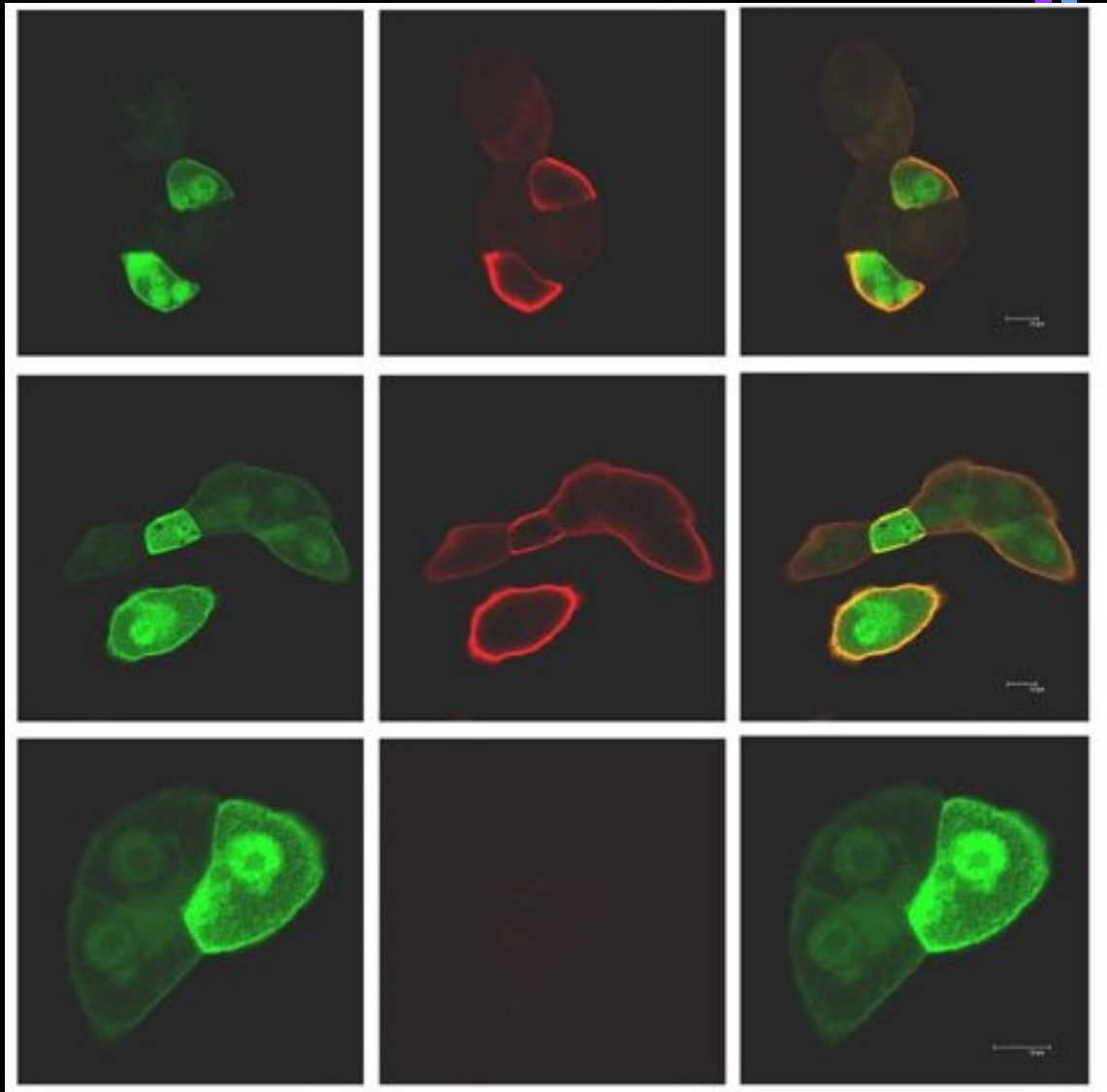
Immuno-fluorescence and RIA

Immuno

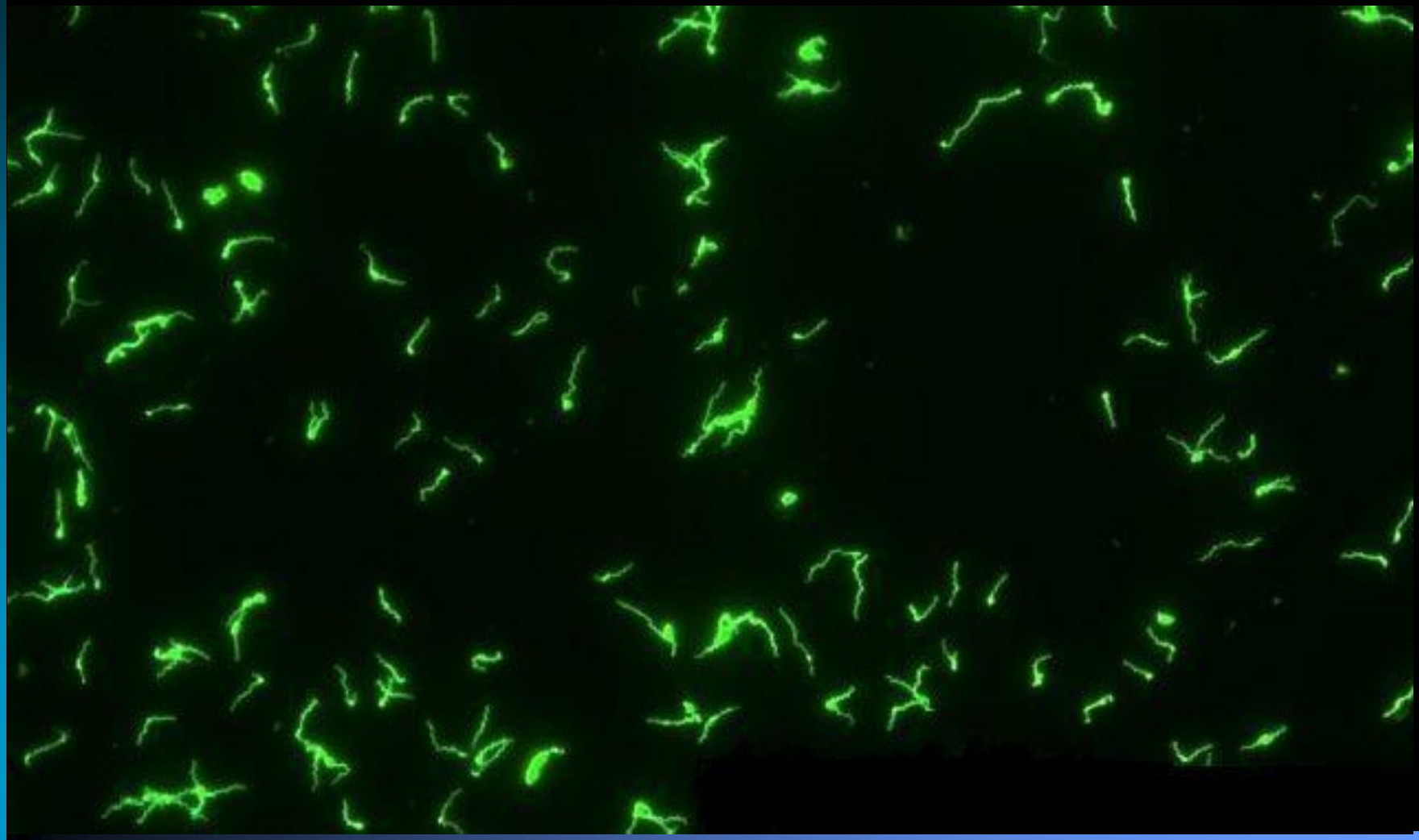


Immunofluorescence

www.amsbio.com



Positive result in both direct and indirect IMF looks the same



Examples of immunofluorescence (diagnostics of *Treponema pallidum*)

Advantage: This reaction uses microscopical slide as surface. This enable us to se the shape of the microorganism.

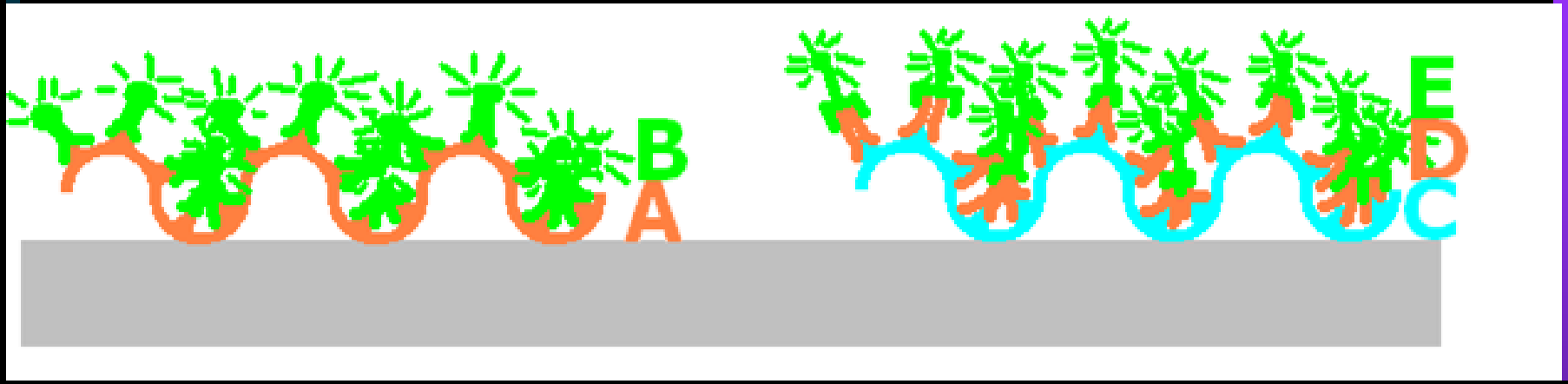
a) Direct imunofluorescence

- (Surface)-(antigen)-(labelled antibody)

b) Indirect imunofluorescence

- (Surface)-(antigen)-(antibody)-(labelled antibody against human antibodies)

Immunofluorescence reaction schemes



A: *Treponema pallidum* – patient`s origin

B: Labelled antibody against *Treponema pallidum* (laboratory)

C: *Treponema pallidum* – laboratory origin

D: Antibody against *Treponema pallidum* – patient`s origin

E: Labelled labor. antibody against human antibody (conjugate)

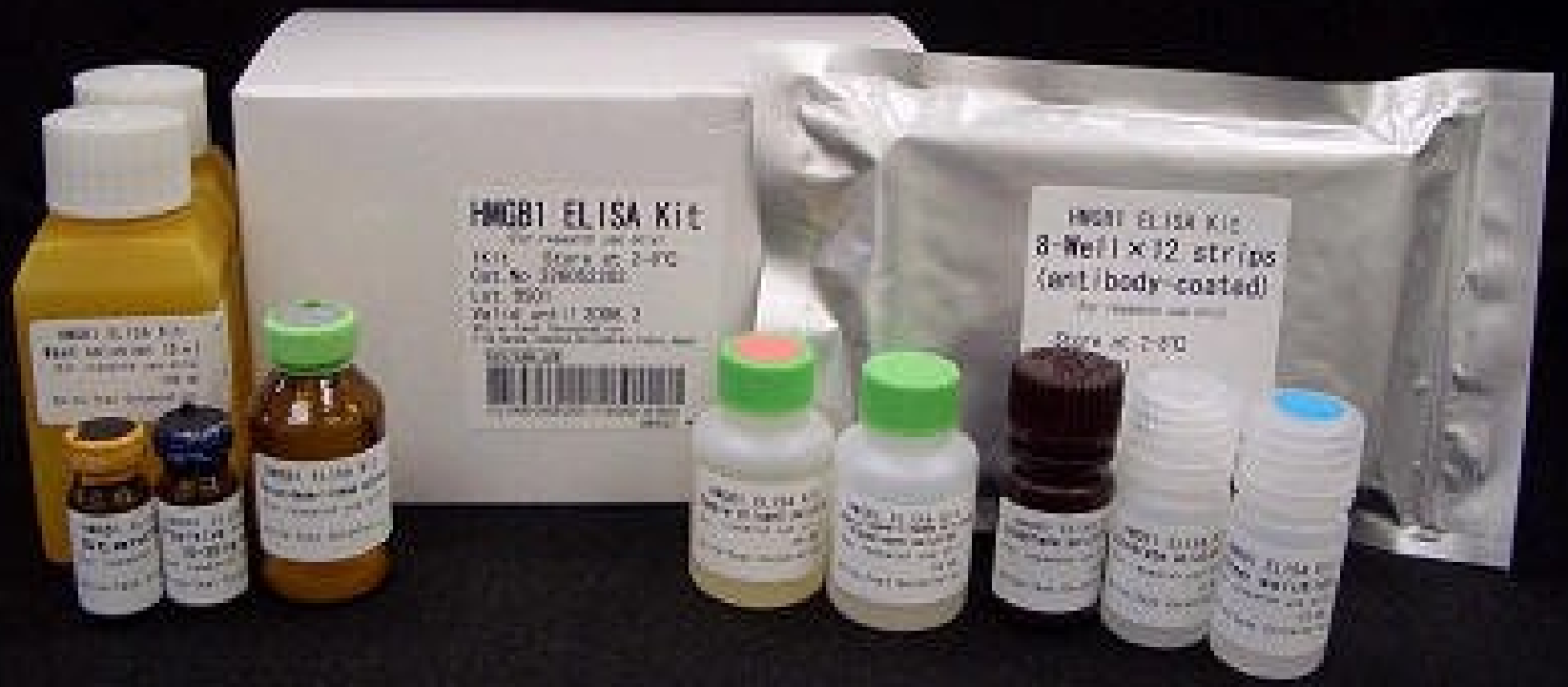
Radioimmunoassay

www.chbr.noaa.gov

darc.tbzmed.ac.ir

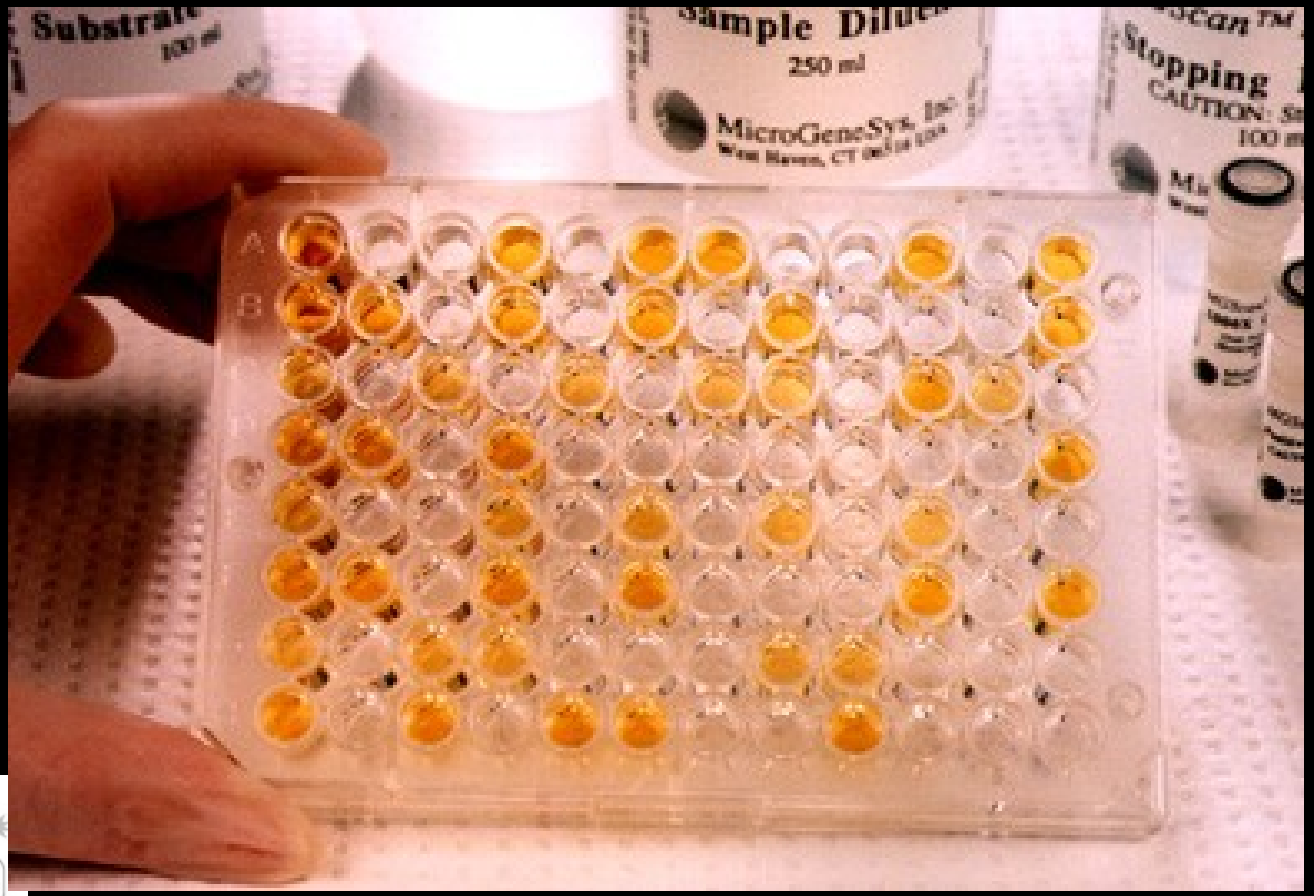
ELISA: principle

ELISA



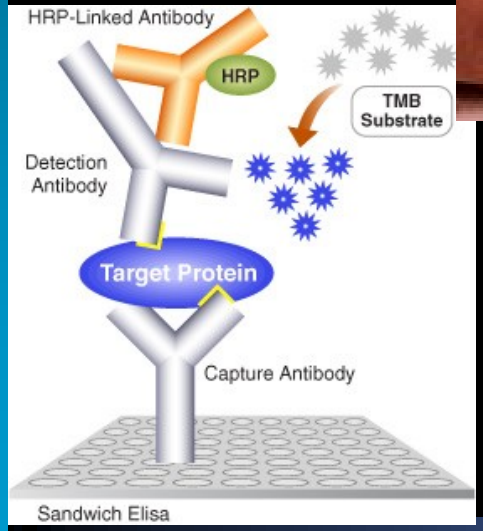
www.genprice.com

ELISA



virology-online.com

www.cellsignal.com



ELISA – why used so much

- In ELISA reaction we have at the end of the whole process an enzymatic reaction. Its intensity is simply described as intensity of colour in a well with the reaction. Very intensive colour = highely positive reaction
- Simplicity for technique and zero radiation is an advantage in comparison with RIA
- Possibility of automatisation is an advantage in comparison with immunofluorescence

Examples of component system

blue = component from specimen taken from pacient‘s body

- Surface-antigen-antibody-labelling agent (D)
- Surface-antibody-antigen-antibody-labelling agent (D, e. g. detection of HBsAg)
- Surface-antigen-antibody-antigen-labelling agent (I)
- Surface-antigen-antibody-conjugate-labelling agent (I)

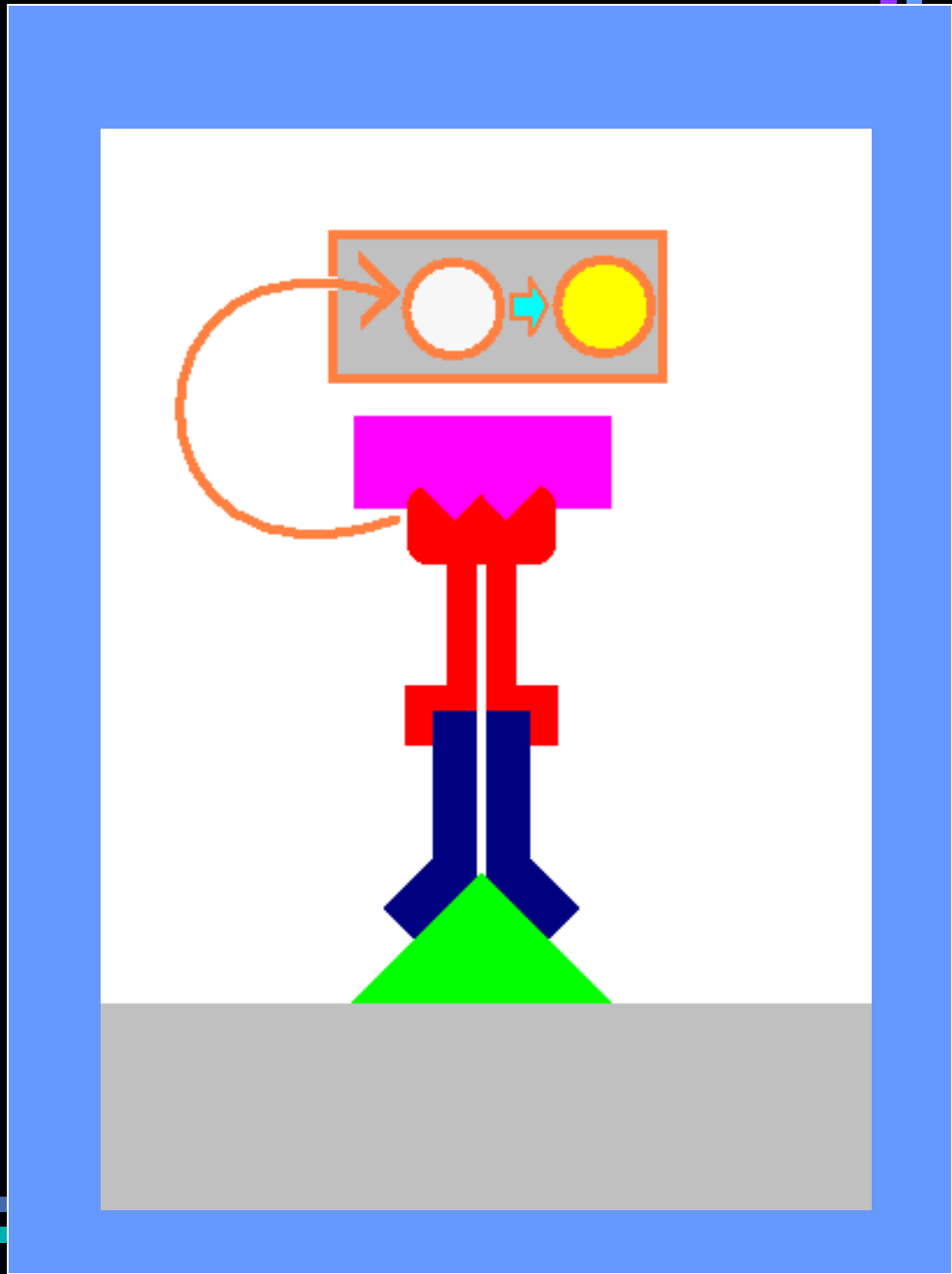
Conjugate is an antibody against human antibody

Importance of the conjugate

- Conjugate is used mostly in indirect reactions (detection of antibodies)
- It is an antibody that has human antibody (e. g. IgM, IgA or IgG) for an antigen
- It can be selective against a certain antibody class
- Use of conjugate is the principle of selective diagnostic of individual immunoglobulin classes

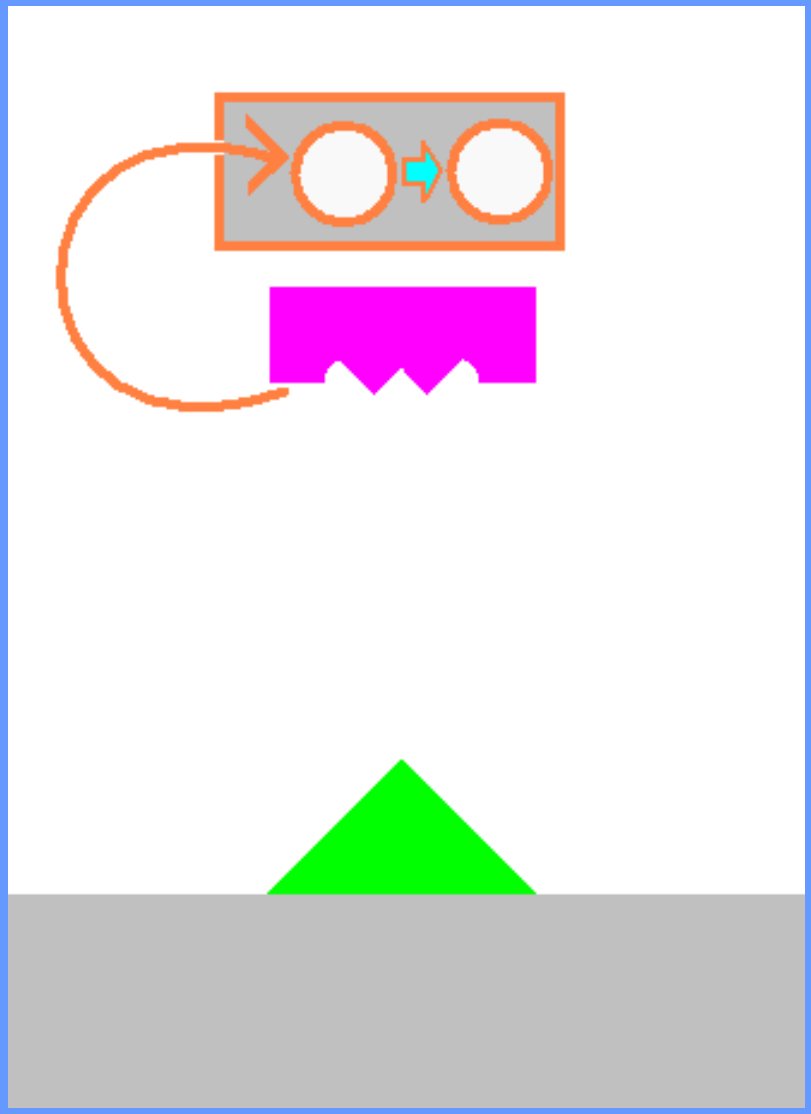
ELISA antibody detection: 1. Positive (searching IgM, IgM present)

All components bind step by step. An enzymatic reaction leads to colour change in the well.



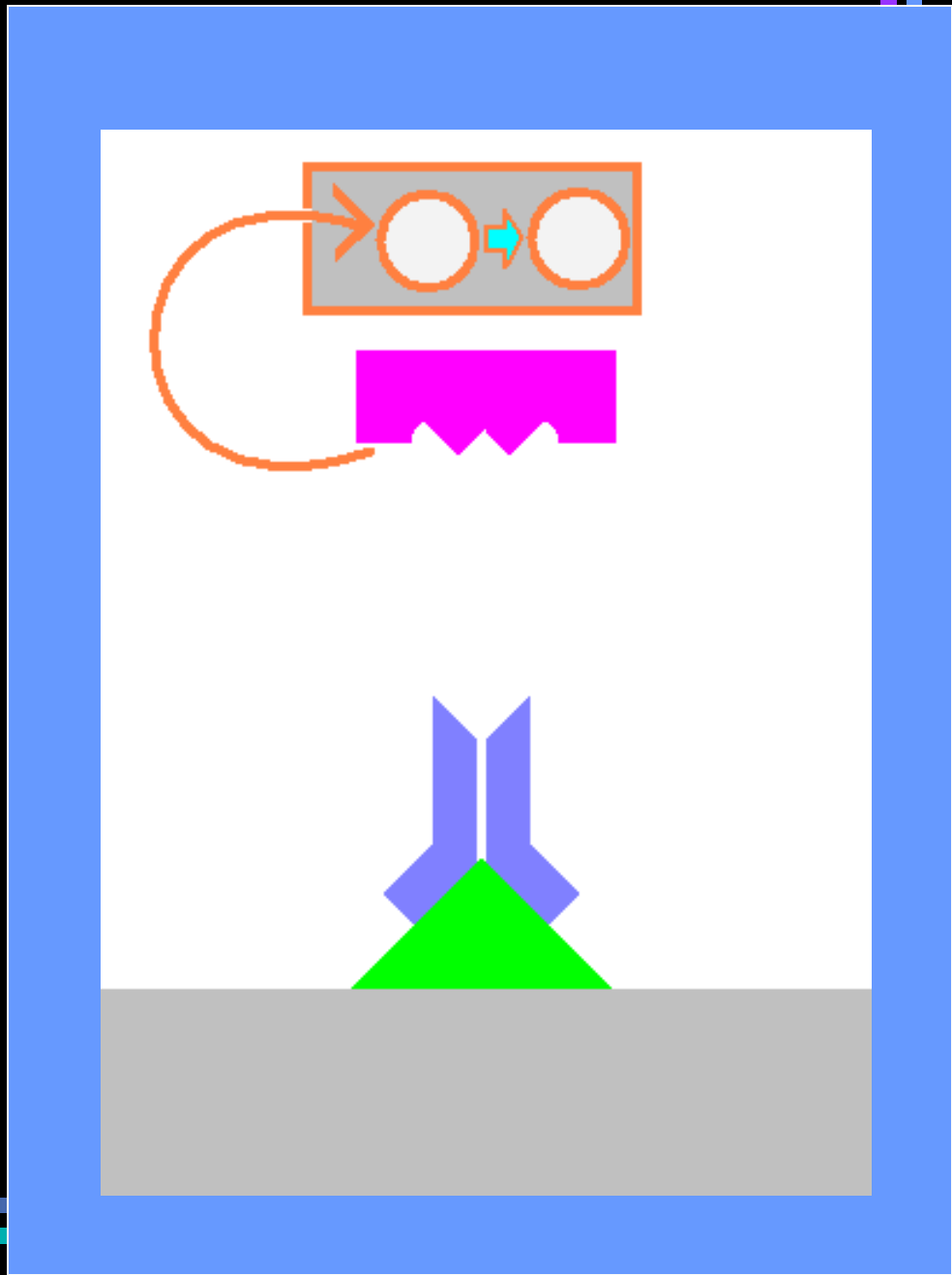
ELISA antibody detection: 2. Negative I (searching IgM, no antibodies)

No antibodies in pacient‘s serum. Conjugate flown out, no change in the well.



ELISA antibody detection: 3. Negative II (searching IgM, IgG present)

Only IgG antibodies in patients serum. Conjugate flown out, no change in the well.



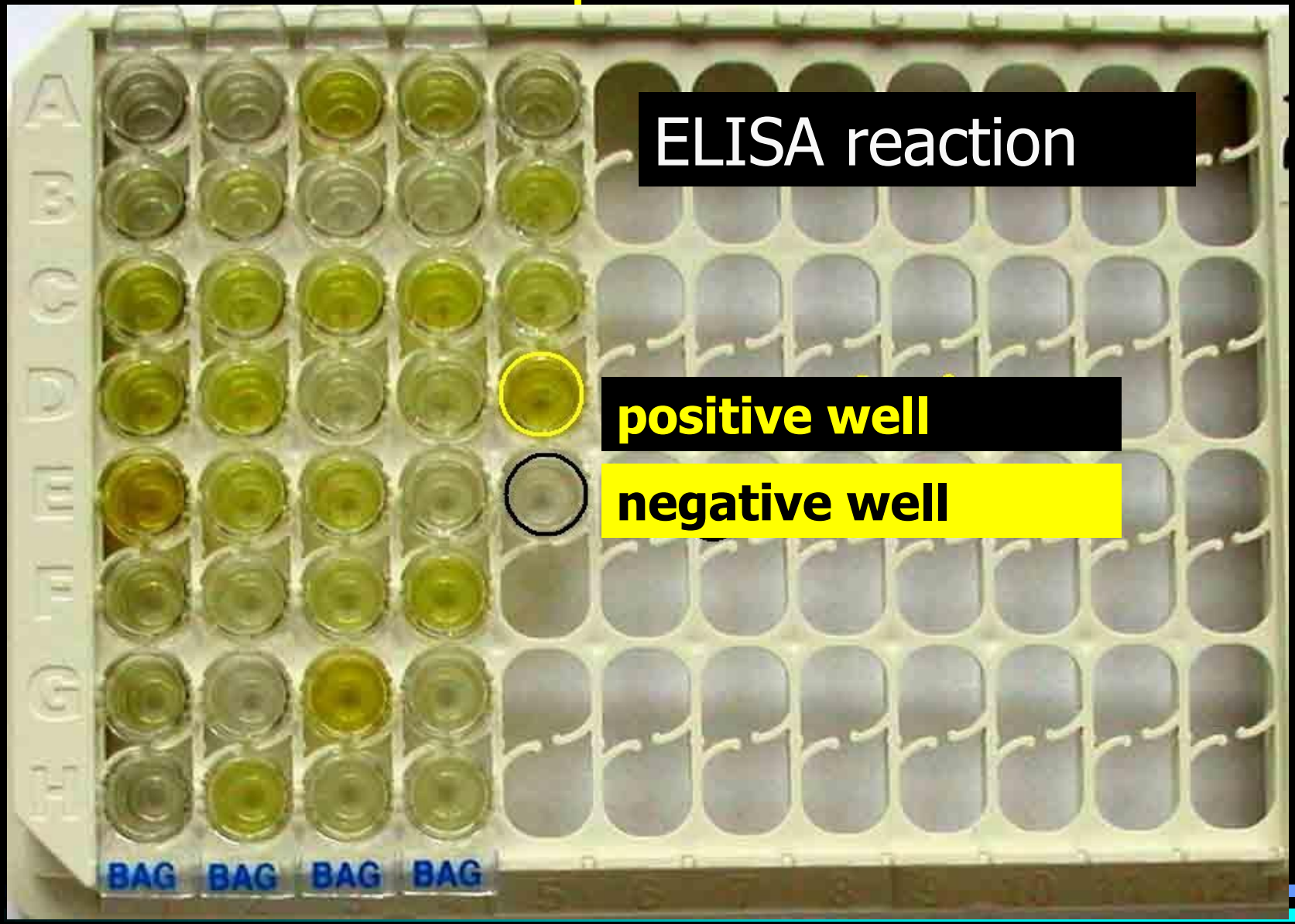
ELISA: practical reading

ELISA – practical description

- Usually we have a microtitration plate. Unlike classical serological reactions, each patient has here not the entire row, but one well only. That is because titers are not assessed.
- First wells, foregoing pacient wells, might be:
  - Bl – blank (for spectrophotometer calibration)
  - K- and K+ – positive and negative controls
  - Cut off (two or three wells) – producer provides „specimens" with just borderline values of absorbance („cutting off" positive and negative results sharp, or plus minus 10 %)

Each ELISA kit is different, like their producers. Some of them do not have any blank. Some of them do not have cut off wells and the cut off is count as average of negative controls + a constant.

ELISA – an example (www.medmicro.info)



Example of ELISA to antigen detection (antigen of *Helicobacter pylori*)

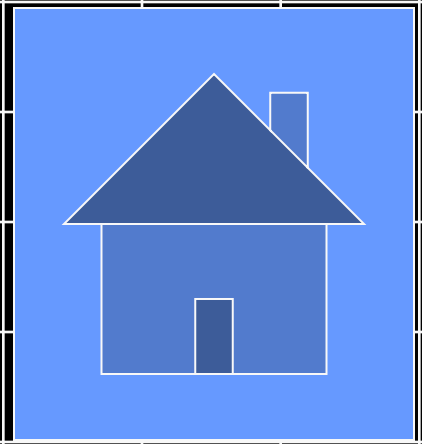
- In ELISA reaction, there is an enzymatic reaction at the end of the process. The intensity corresponds with the colour intensity in the well.
- Collour intensity can be evaluated spectrophotometrically
- As positive we consider values higher than reference given „cut off"
- *Usual principle: Surface-antibody-antigen-antibody-enzyme-substrate*

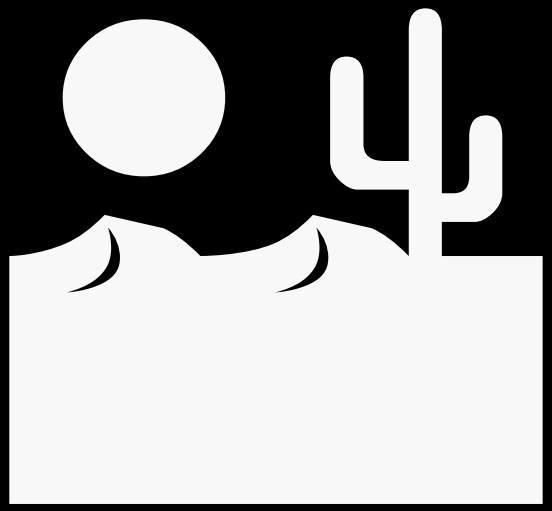
Example of ELISA to antibody detection

- In indirect antibody detection using ELISA usually IgM and IgG are assessed separatelly
- In our case, IgA is used instead of IgM
- Values higher than „cut off" are again considered to be positive
- Often we have a „borderline field". For example, results ranging 90 % to 110 % of cut off vallue are described as „borderline", below 90 % as „negative", above 110 % as „positive"
- *Common principle: Surface-antigen-antibody-conjugate-enzyme-substrate*

Example of an ELISA scheme to antibody detection

BL	4	BL	4
K-	5	K-	5
K-	6	K-	6
K+	7	K+	7
K+	8	K+	8
1	9	1	9
2	10	2	10
3	11	3	11
IgA		IgG	





Western blotting

Western blotting

- *Language joke (researcher Southern)*
- In fact, it is an ELISA, but antigenic mixture is divided electroforetically to individual determinants
- So it is more precise, and helpfull mostly in situations, where classical ELISA is problematized by cross-reactions

Western blotting – principle

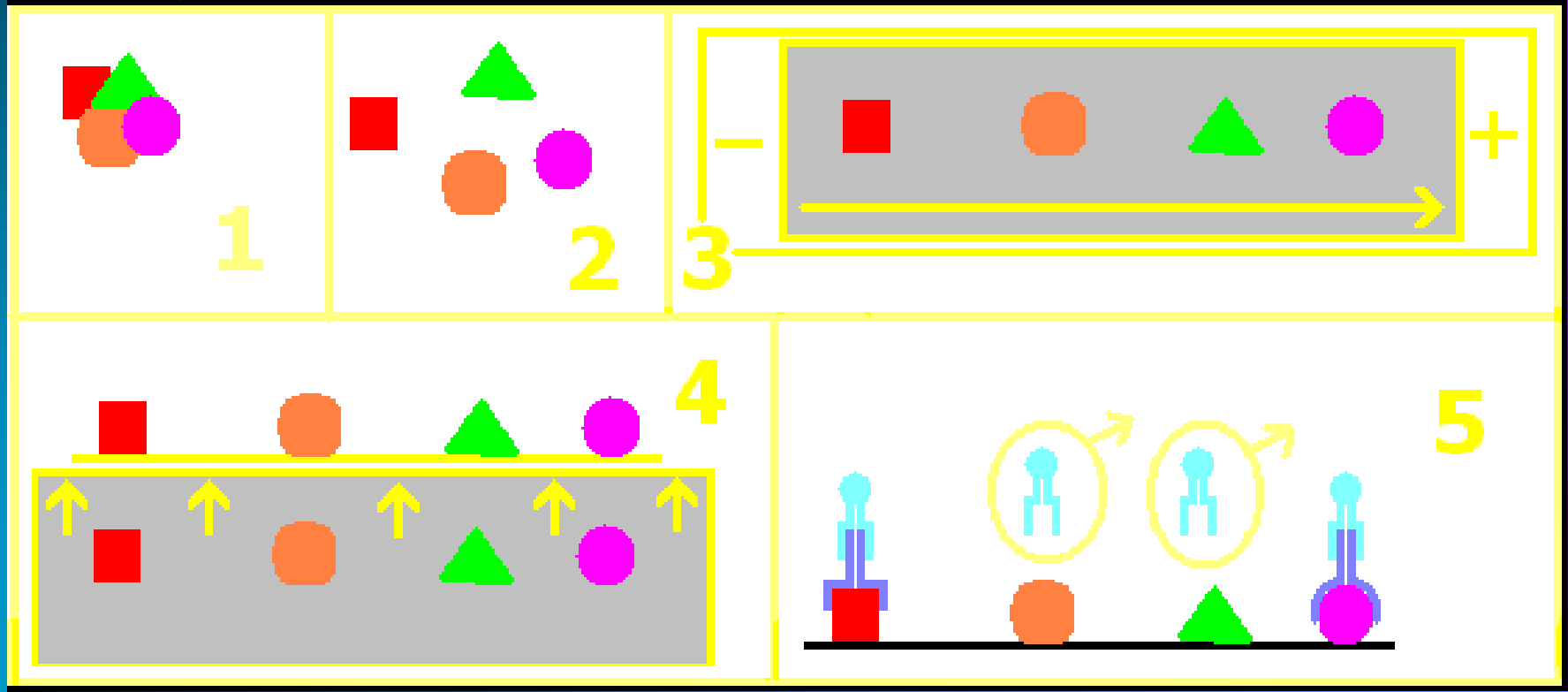
1: original antigen (mixed)

2: decomposition of antigen by a detergent

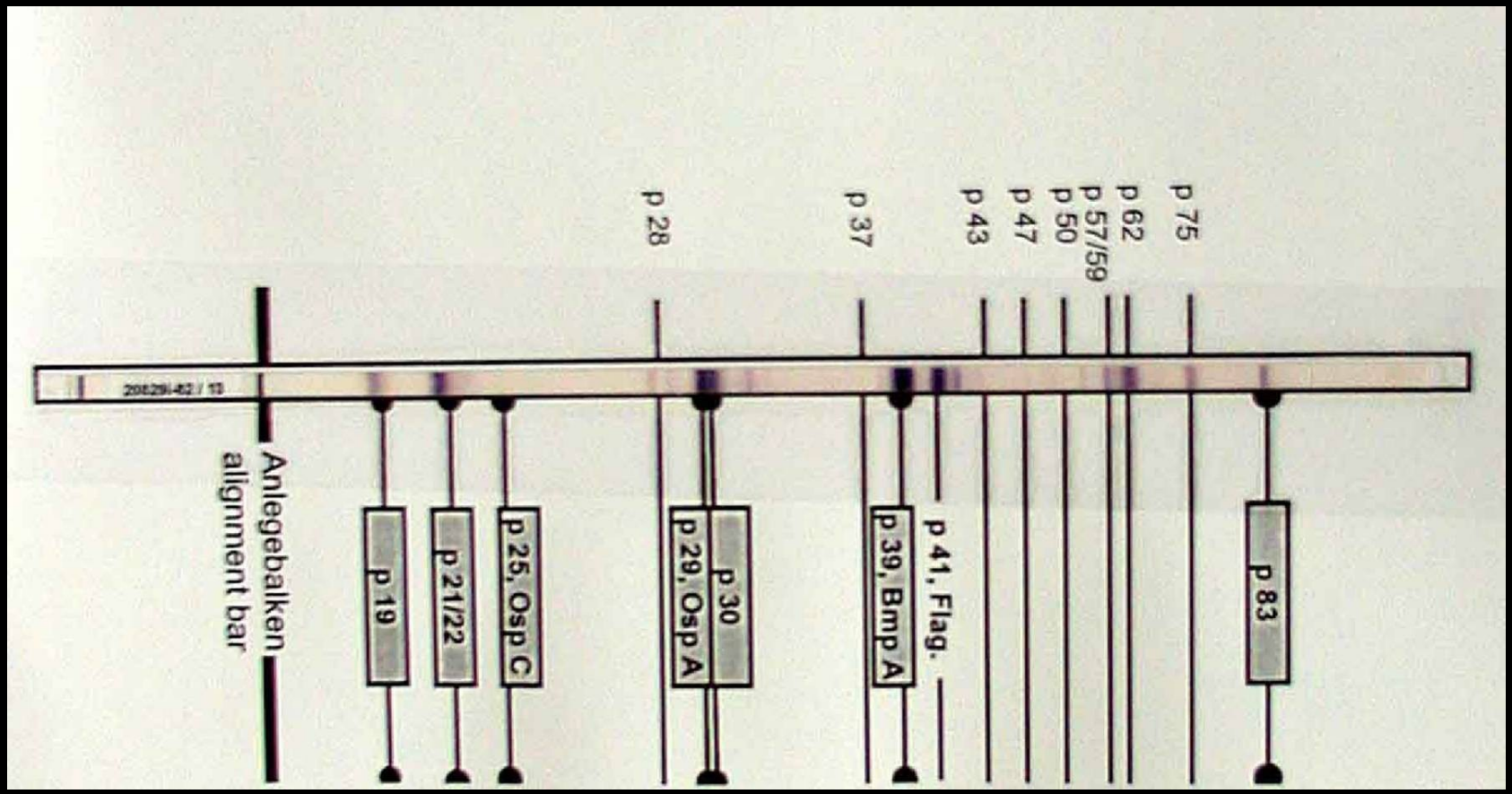
3: electroforetic division of antigen

4: „blotting" of divided antigen to a nitrocelulose membrane

5: ELISA reaction (only some antibodies present)



Western blot – example
(picture from www.medmicro.info)



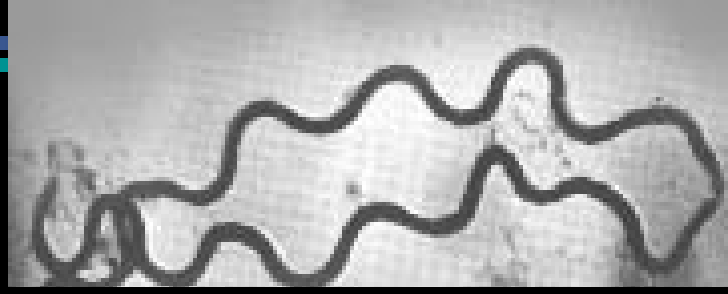
Example of reading of a Western blot (in Lyme borreliosis)

- Presence of **at least two specific bands** (labelled on a pattern) → assessed as positive
- Exceptions:
  - **in IgG** positivity of one band is sufficient, if it is *vlsE* band (it is highly specific)
  - **in IgM** positivity of one band is sufficient, if it is *ospC* band (it is highly specific)

The End

(A picture name: Antibody)

www.twitchfilm.net/archives/003401.html



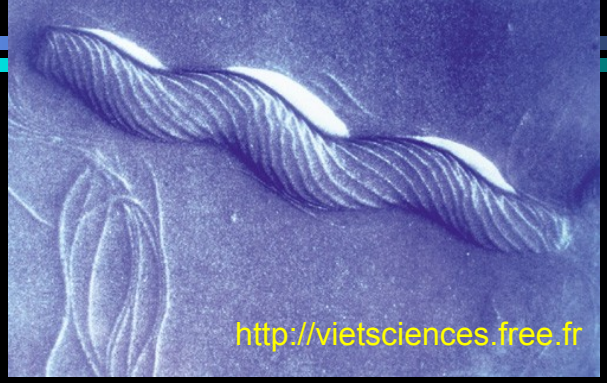
Treponema pallidum

- A spirochet, causing syfilis
- Syfilis is a classic sexual disease. It is transmitted sexually only. But it is a systemic disease – in developped stages the whole body is affected (gummata, aortal dissection, neurolues, psychical symptomas)
- Some subspecies of *T. pallidum* and some other treponemas cause other diseases (framboesia – yaws, *T. pertenue*)
- Some treponemas are non-pathogenous

Borrelia burdorferi sensu lato

- Another spirochet, causing Lyme disease (after town of Old Lyme in the U. S. A.) and subclassified into *Borrelia burgdorferi* sensu stricto (= „strict sense of meaning“), *Borrelia garinii*, *Borrelia afzelii* and others
- The disease is characterized by so named erythema migrans and then usually more symptomas follow
- While in the USA mostly *B. b.* sensu stricto is common and joint symptomatology is common, in Europe *B. garinii* and *B. afzelii* are more common, and the typical disease is neuroborreliosis
- Besides lyme diseases there exist other species causing recurrent fever (*B. duttoni, B. recurrentis*)

Helicobacter pylori



- Peptic (= gastric / duodenal) ulcus is caused by more causes. Such diseases are called multifactorial diseases.
- The part of *Helicobacter pylori*, a spiral rod (not spirochet!), on ulceral disease is still discussed, not only among GPs, but even among specialists. Even healthy persons may have a helicobacter in their stomach. Nevertheless, certain and not negligeable role of this pathogen is sure.