

Searching for microbes Part IX.

Neutralisation reaction

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To practical of VLLM0421c

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Content of this slideshow

Neutralisation reaction – principle

Individual neutralisation reactions

ASO and its importance

Tale

- Once there was a killer toxin, and the toxin wanted to kill a red blood cell
- That toxin had in also character of an antigen, that challenges the body to produce antibodies
- And when the **toxin prepared for killing the RBC**, an **antibody**, crossed his way, bound to it and **did not allow him killing**
- The red blood cell was very happy, and it sedimented to the bottom with other RBCs.

What to learn from the tale

- Today, we have **neutralisation reaction**
- This reaction is important in **viruses** and **bacterial toxins**, that can be **directly neutralized** by a corresponding antibody
- The whole bacterium is rarely neutralized like that
- Majority of neutralisation application is **in virology**. An exception is the most common serological reaction at all – **ASO reaction**

Neutralisation reaction – principle

Neutralisation reaction: general principle

- There are many ways, how antibodies do work. One of them is direct neutralising effect
- This effect is rarely present in whole bacteria. On the other hand, it may be observed in whole viruses, and in bacterial toxins

Nevertheless, sometimes antibodies neutralise some characteristic of the whole bacteria, e. g. motility of Treponema in Nelson's test

Neutralisation schematically

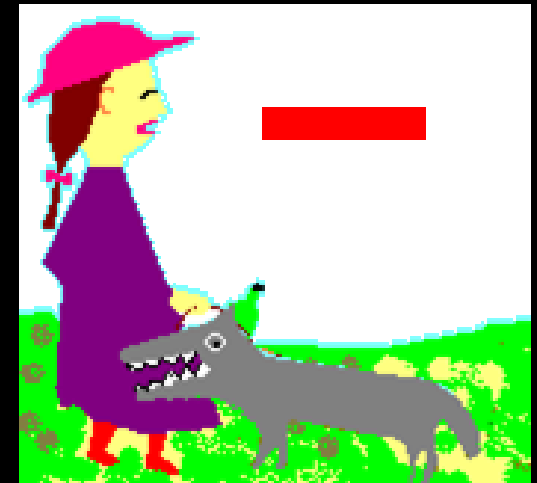
- Antibody (Ig) prevents an effect of a toxin/virus to a cell / red blood cell



Cell in a tissue culture or a red blood cell

Antibody

Toxin or virus

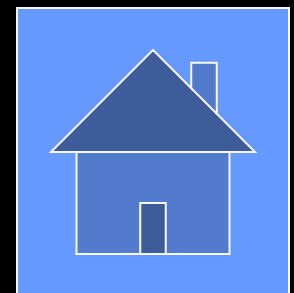


Cell in a tissue culture or a red blood cell

Toxin or virus

Examples of neutralisation reactions

Task	Neutralised	Object	Reaction
1	Bacterial toxin (haemolysin)	RBC haemolysis	ASO
2	Virus	RBC agglutination	HIT
3	Virus	Cell metabolic effect	VNT



Individual neutralisation reactions

ASO

- **Principle:** The antibody blocates the haemolytical effect of the toxin (streptolyzin O) on the RBC. Positive is blocation of haemolysis (as in CFT, but for a different reason)
- **The microtitration plate** is composed of a positive control and seven patient
- **The titer above 250** is supposed to be risky for an autoimmune disease.

Course of serum dilution – ASO

Common course (dilution with reometric row, coefficient 2) would be too rough, we need a more detailed one. In fact, it is a geometric row too, but the coefficient is 1,2 and not two as usually

Hodnocení výsledků ASLO

řada	1	2	3	4	5	6	7	8	9	10	11	12
hodnota ml.l.	100	120	150	180	225	270	337	405	506	607	759	911

HIT

- **Haemagglutination Inhibition Test:** Pay attention, it is NOT an agglutination reaction, it is a neutralisation! Antibody neutralises the aggregation of RBCs due to viruses.
- **So: Potato-like shape = negative response. Dense round target = positive response**
- **Example of use:** We can read HIT results for tick-borne encephalitis. In each patient an acute and a convalescent serum is evaluated

Interpretation of acute vs. convalescent sera is of course the same as in any other serological reaction

Remember:

- HIT is not an agglutination reaction, it is neutralisation of viral agglutination
- HIT differs from ASO reaction mostly by the fact, that **the RBCs are not haemolyzed, but agglutination**. But the fact, that a specific antibody blocates the reaction is valid in both of the
- HIT for detection of antibodies against tick borne encephalitis (unlike ASO) is again a typical „indirect diagnostic“


HIT for tick-borne encephalitis: example of a clinical situation

- We have several patients with **suspicion for tick borne encephalitis**, already tested using complementfixing test (see in J08 practical)
- Now we have decided to use an **independent test** to check the results

VNT (do not confuse with TNT ☺)

- Virus Neutralisation Test
- Cell culture uses to be damaged by a virus. The damage is visible as a change of colour from original yellow to changed red (pH is changed)
- Antibodies, if present, may prevent this viral action on the cell culture, so the colour remains yellow
- Titre = last well with unchanged colour

VNT – clinical situation

- Patient R. S., 35 years, has **chronical pain in chest**. Cardiological examination showed suspicion for inflammation of heart muscle (myocarditis)
- As **coxackieviruses**  are common causative agent of myocarditis, it was decided to perform test of antibodies against these viruses

VNT – example of use in coxsackieviruses

- The whole panel belongs to one patient examination. Odd rows = acute serum, even rows = convalescent rows. Every two rows = one coxsackievirus (B₁ to B₆)
- First column has dilution 1 : 5 (then 1 : 10, 1 : 20...)
- Last column = controls. When there are six yellow and six red wells here, everything is OK.
- **Titre is the last well with unchanged (yellow) colour.**
- When two coxsackieviruses have a significant (at least four-fold) increase of titer, it might be a co-infection, **but it is more likely that the coxsackievirus with the lower titre has a cross-reaction only**



ASO and its importance

What is the antistreptolysin O and why we attempt to detect it

- After every streptococcal infection antibodies are produced, often including antibodies against streptococcal toxin – streptolysin O.
- Nevertheless, sometimes after infection the antibodies increase instead of decreasing. Antibodies are bound to some structures of the host organism (autoimmunity), so a „circulus vitiosus“ starts to run
- In such a situation, paradoxically the antibodies are worse than the pathogen that challenged the antibody response to protect us.

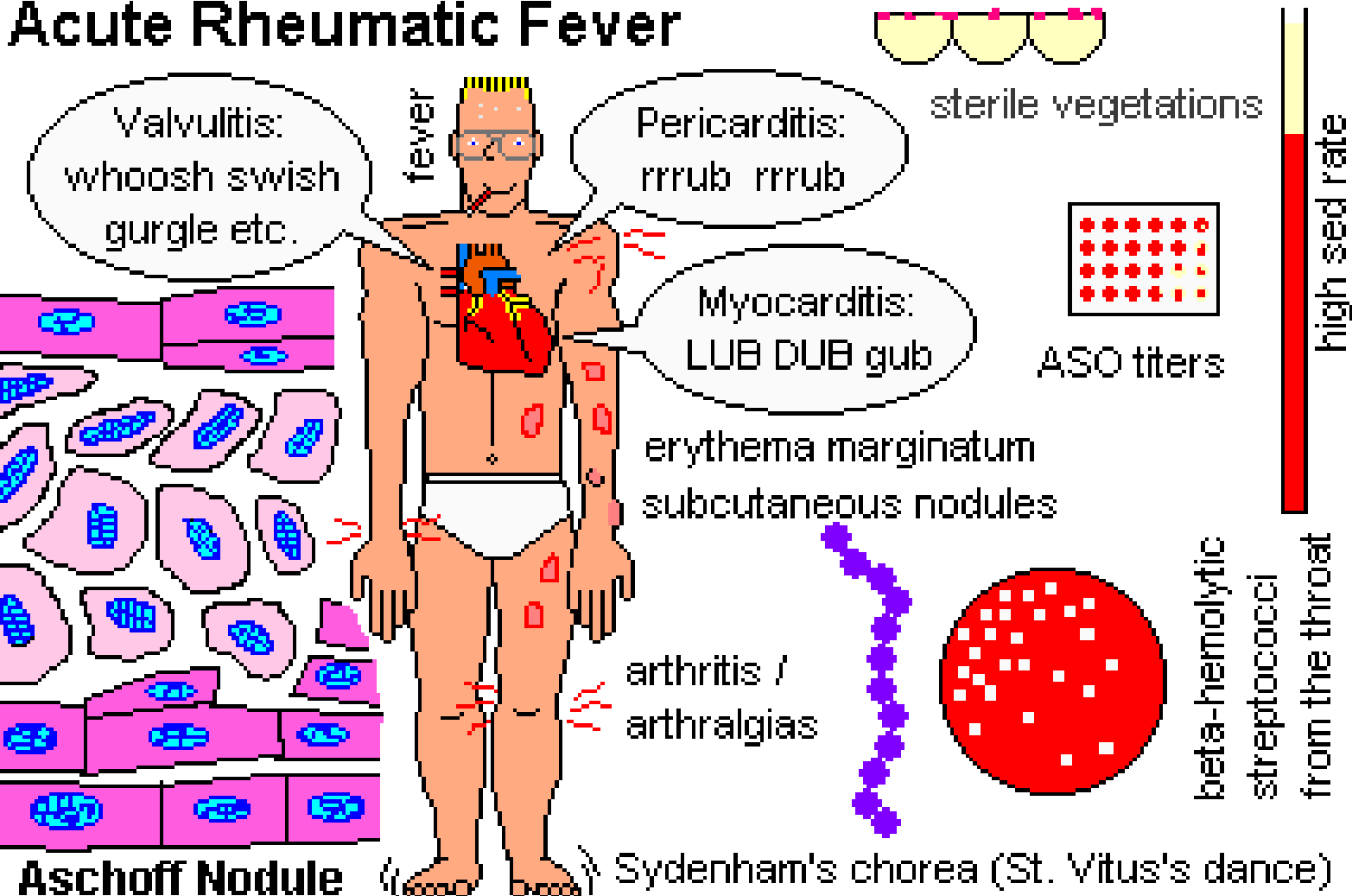
Remember:

- ASO is not an indirect diagnostics reaction, despite the fact that we search for **antibodies**. The aim is not to get a pathogen, but to assess the antibodies themselves, as they may be dangerous
- Indication for ASO examination: suspicion for so named „late sequellae“ of streptococcal infection: acute glomerulonephritis, or rheumatoid fever

Rheumatic Fever

<http://mednote.co.kr>

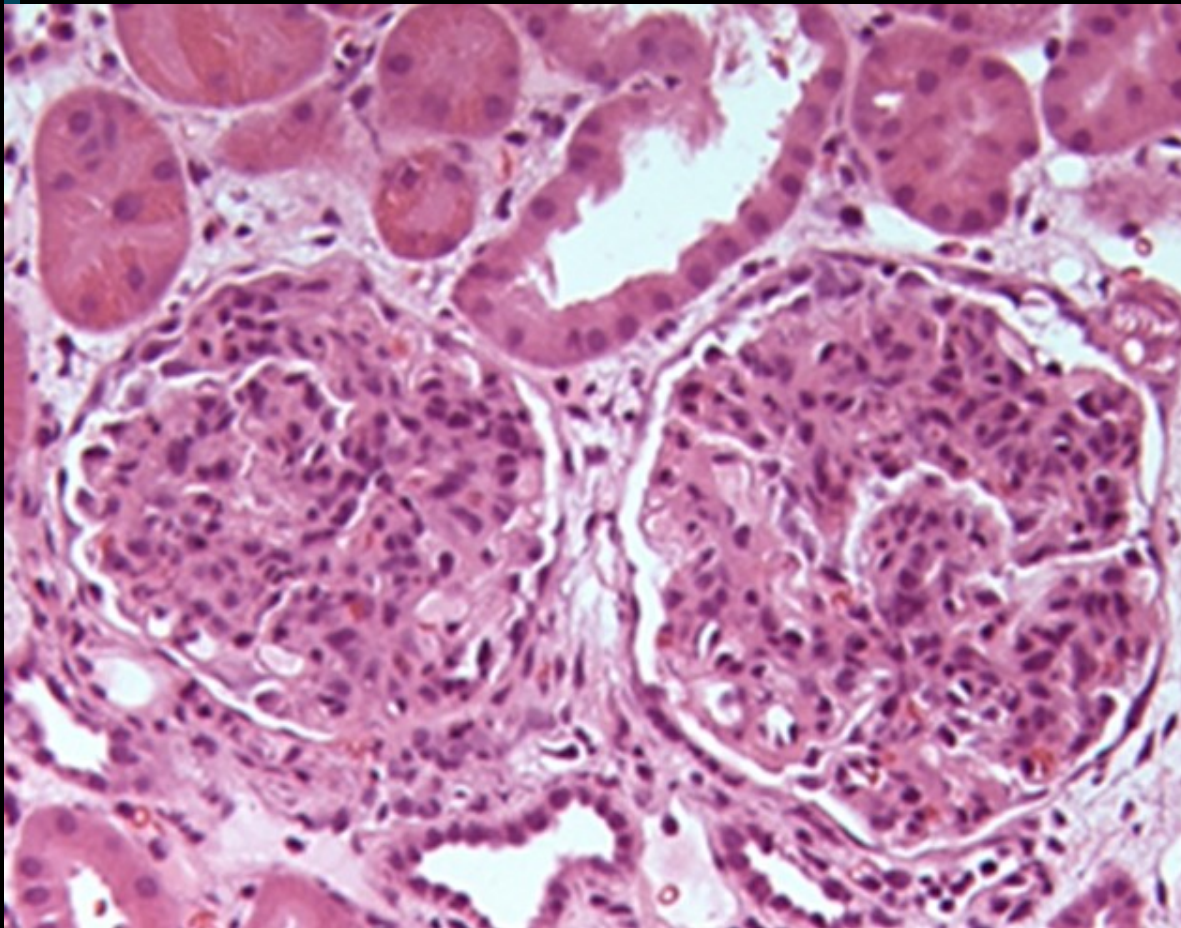
Acute Rheumatic Fever



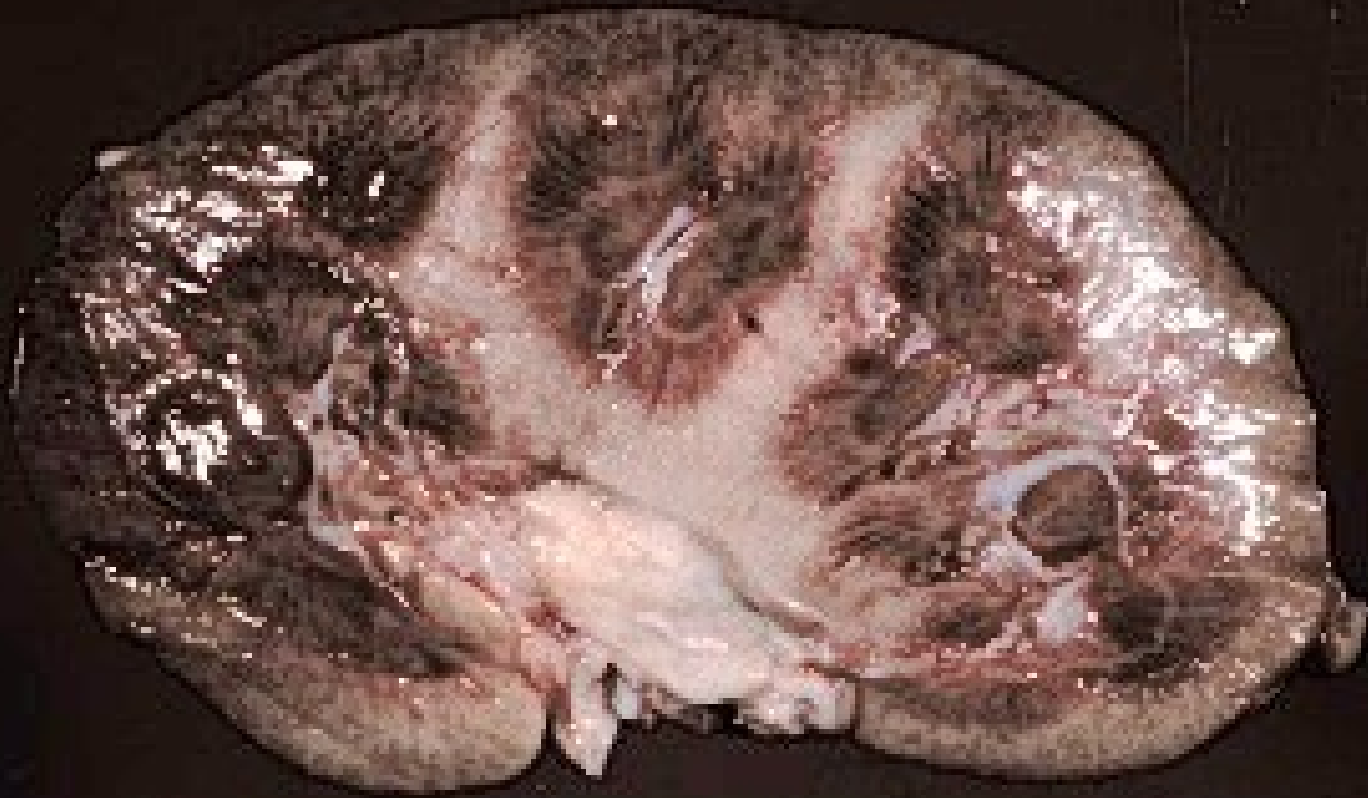
Accute glomerulonephritis

Diffuse inflammatory cellular infiltration and mesangial hypercellularity (Hematoxylin and Eosin Staining: original magnification X 200)

www.ispub.com

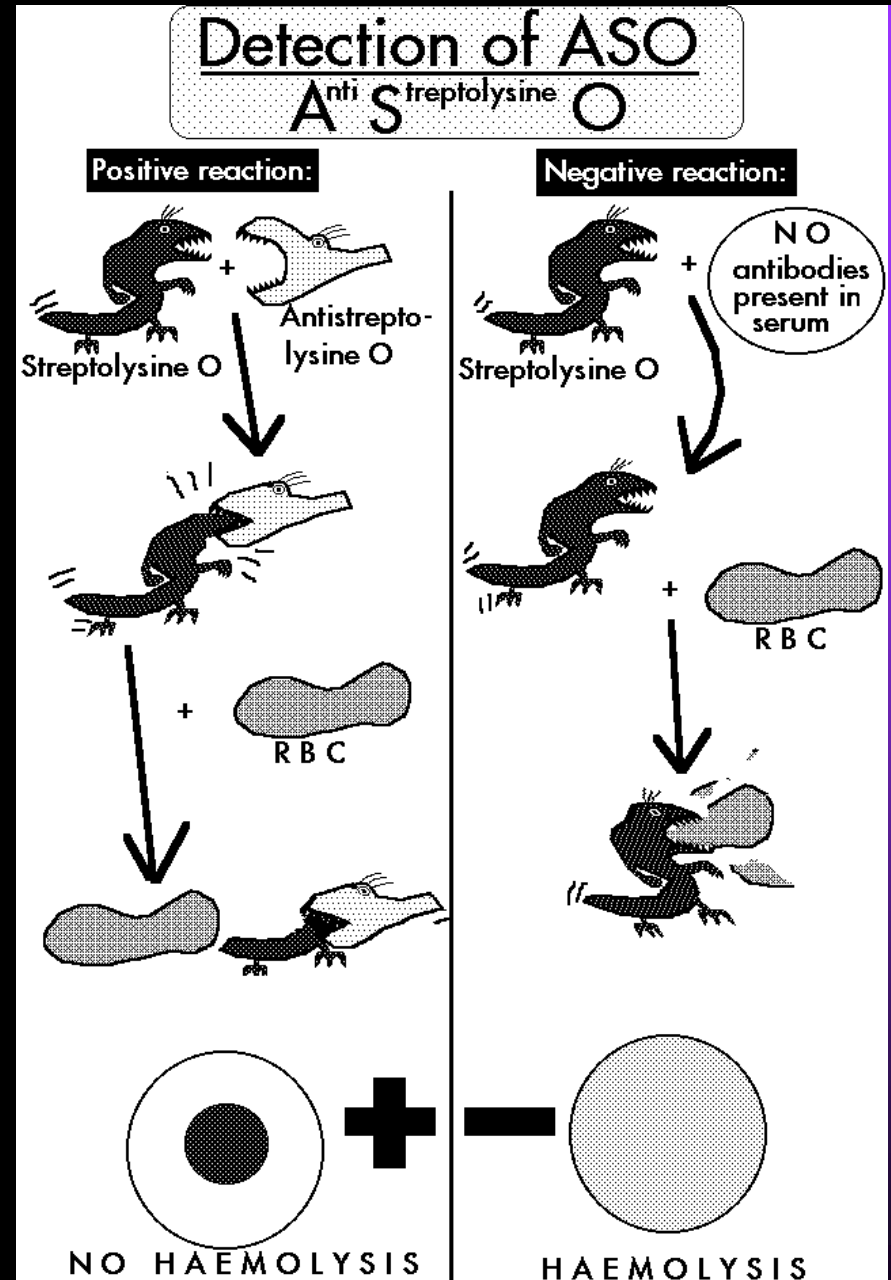


Acute glomerulonephritis II

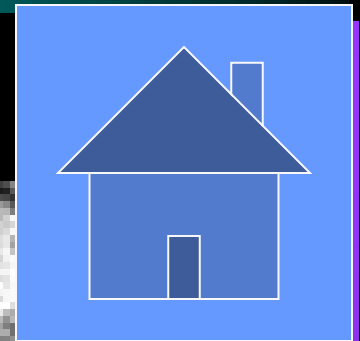
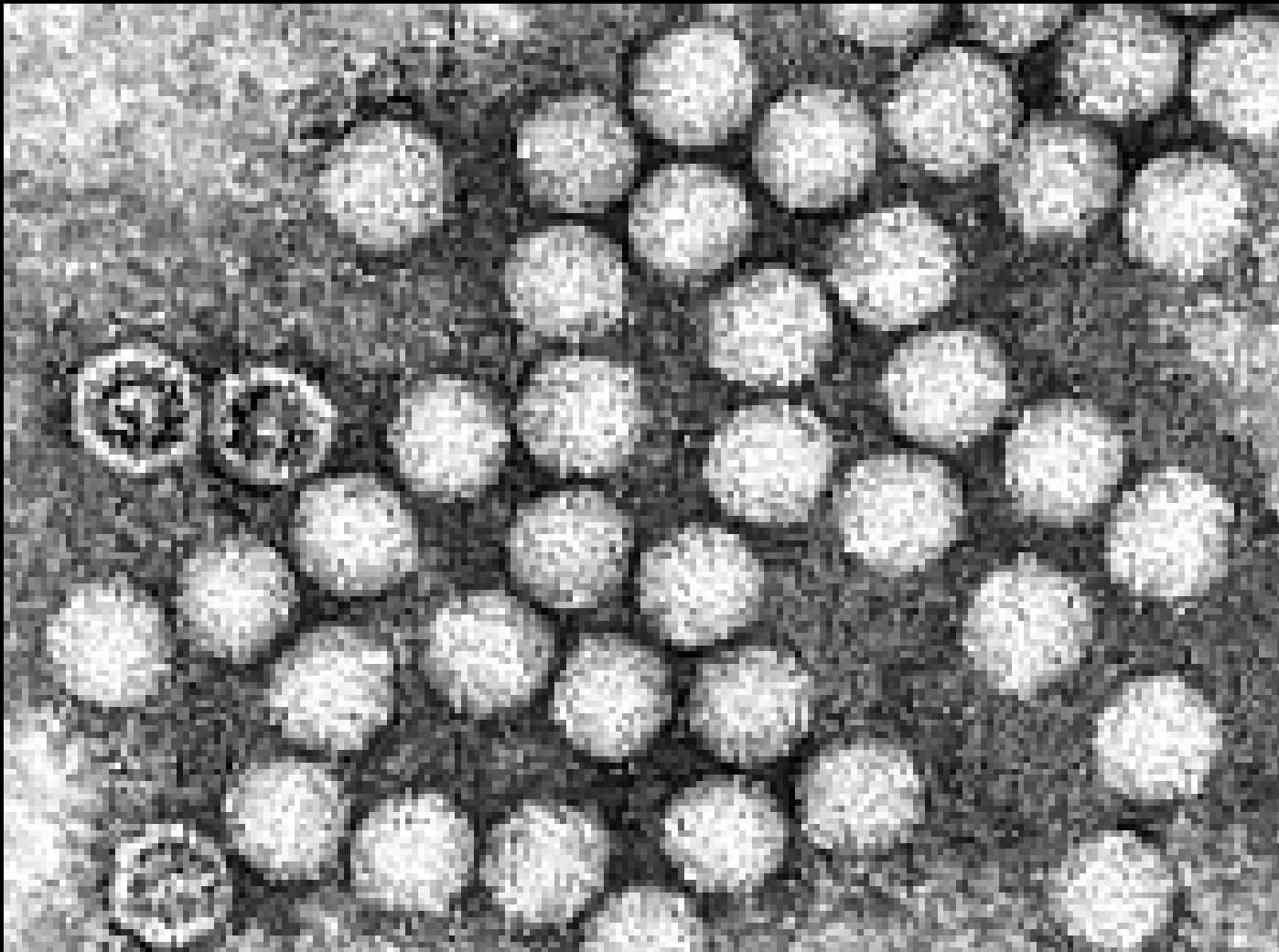


ASO examination principle: haemolysis neutralisation

In Czech Republic, abbreviation ASLO is used for the same thing as ASO in English



The End



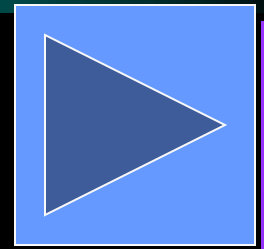
<http://web.uct.ac.za/depts/mmi/stannard/emimages.html>

Coxsackieviruses: survey of family *Picornaviridae*



- Family *Picornaviridae* contains mostly following viruses important for humans:
- **enteroviruses**, (name shows their way of transmission, but they cause infection mostly outside intestine!) further classified into
 - **polioviruses** – viruses of poliomyelitis
 - **coxsackieviruses** and **echoviruses**
 - **newer enteroviruses 68, 69, 70 and 71**
- **rhinoviruses** – viruses of common cold
- **virus of hepatitis A**

Coxsackieviruses – more info



- There exist coxsackieviruses A1–A22, A24 and B1–B6
- **Diagnostic** can be done by virus isolation on newborn mice or tissue cultures
- **Indirect diagnostic** is difficult because of cross-reactions; nevertheless, it is used in coxsackieviruses of B group in suspicion for myocarditis

Coxsackieviruses – pathogenicity

- **CNS:** aseptic meningitis (majority of types)
- **herpangine** (A types, mostly A4)
- **hand-foot-mouth disease** (A16)
- **respiratory infections** (all types)
- **myocarditis and other muscle disease** (B types)
- **lymphadenitis** (all types)
- relation of some types of **diabetes mellitus** (B group)?

