

Searching for microbes Part VIII.

Complementfixing test

Ondřej Zahradníček

To practical of VLLM0421c

Contacts to me:

777 031 969 zahradnicek@fnusa.cz

ICQ 242-234-100

Content of this slideshow

Dynamics of titre

Complement and its properties

Complement fixing test (CFT), its principle

Trouble shooting in CFT

Examples of CFT use in practice

Tale

- There was a curious park guard.
- He wanted to know the true relations between a boy and a girl that used to visit his park. **Are they a couple, or they aren't?**
- He knew, there is **one only bench in the park**. When one wanted to embrace somebody else somewhere, one had to do it there.
- So, he placed parts of a plant (globules with hooklets, see next slide) on the bench with hope, that the couple would catch them on their clothes

The plant



<http://www.ordinace.cz/clanek/lopuch-vetsi-lopuch-plstnaty/>

However – how to ascertain...

- ...when both the girl and the boy used another exit?
- Then the guard realized, that during a moment **his niece and her boy-friend will come to him**, and he was sure, that on the way through the park, **they will certainly use the bench for embracing.**
- And so he made a plan: **when his niece and her boy-friend will have globules on them, it means, that the first couple was no true couple, as it did not catch the globules first.**

What to learn from the tale

- Today we have to learn complement fixing test, quite a complicated test.
- Not only that we use **complement** to visualise antigen-antibody complex, but also two more parts of the reaction: the **indicator couple** (niece and boy-friend).
- This couple consists of **indicator antigen (sheep RBC)** and **indicator antibody (amboceptor = rabbit antibody against sheep RBC)**

Dynamics of titre

Interpretation of serological reactions

- **Antigen detection:** it is a direct method. Positive result means presence of the microbe in the patient's body
- **Antibody detection:** it is an indirect method. Some ways how to assess, when the microbe met the body:
 - Amount of antibodies (titre) and mostly **its changes during the time (dynamics)**
 - Class of antibodies: IgM/IgG (More in J10)
 - (*Avidity of antibodies*)

Dynamics of titre

1 first patient's visit

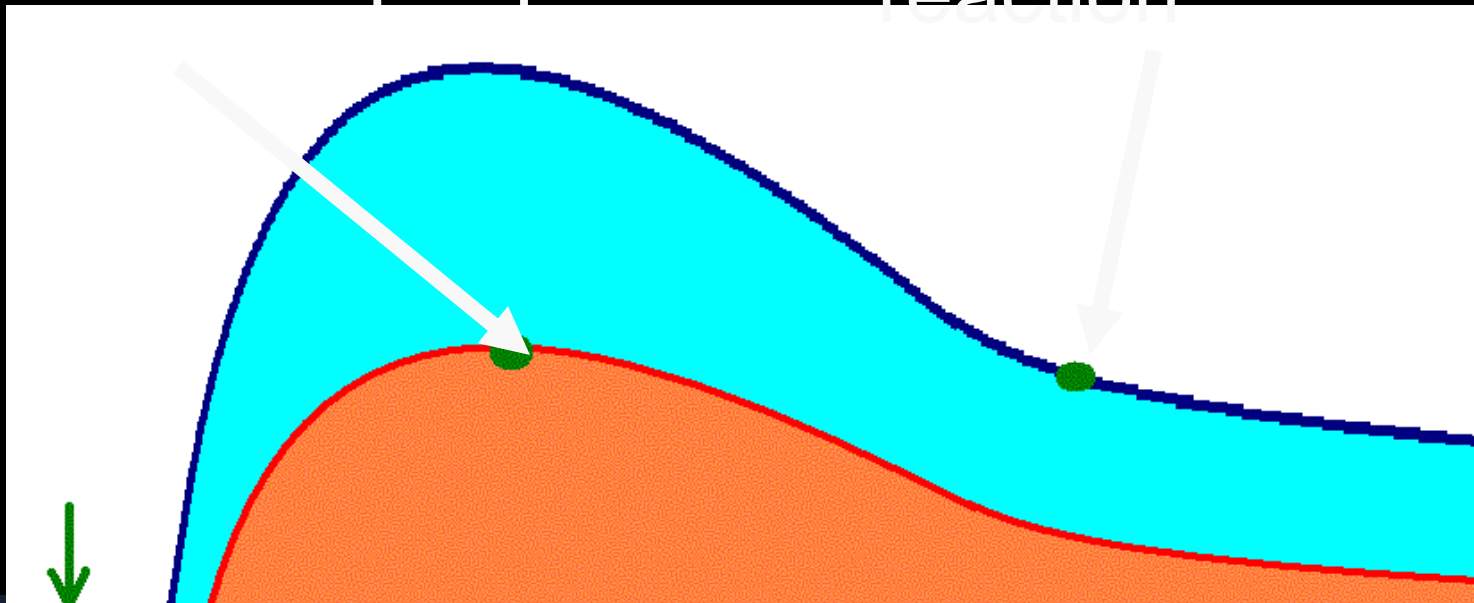
2 after 2 – 3 weeks

- **Absolute amount of antibodies** is not the most sure information: some patients are poor antibody-producers, etc.
- **Dynamics of titre:** better, means how the response gets changed during the time (usually during two or three weeks)



Why the titer alone is not sufficient

- Sometimes a patient with low reactivity has a low titre even in
- On the contrary, a very reactive patient has a high titre long after the reaction



Pair sera and non-pair sera

- **Pair sera** = first specimen is kept in the refrigerator until the second comes to the lab (cca 10–14 days), and then examined together. **4-fold increase** is told to be significant under such circumstances
- **Other situations** (second specimen is examined separately): an accidental error should be taken into account. So, usually at least **8-fold increase** is needed

Dynamics of titre – more aspects

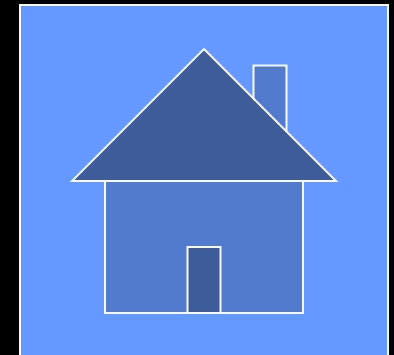
- A special situation is so named **seroconversion** – there are no antibodies in the first specimen (it is too soon), but there are antibodies in the second one. Such a finding is more sure than the four-fold increase

- **Sometimes titer decrease is found instead of increase** (a subacute infection)

- ***Titre value does not correspond to infection activity. Often the highest amount of antibodies comes after the end of disease.***

Examples of various effects of titre dynamics:

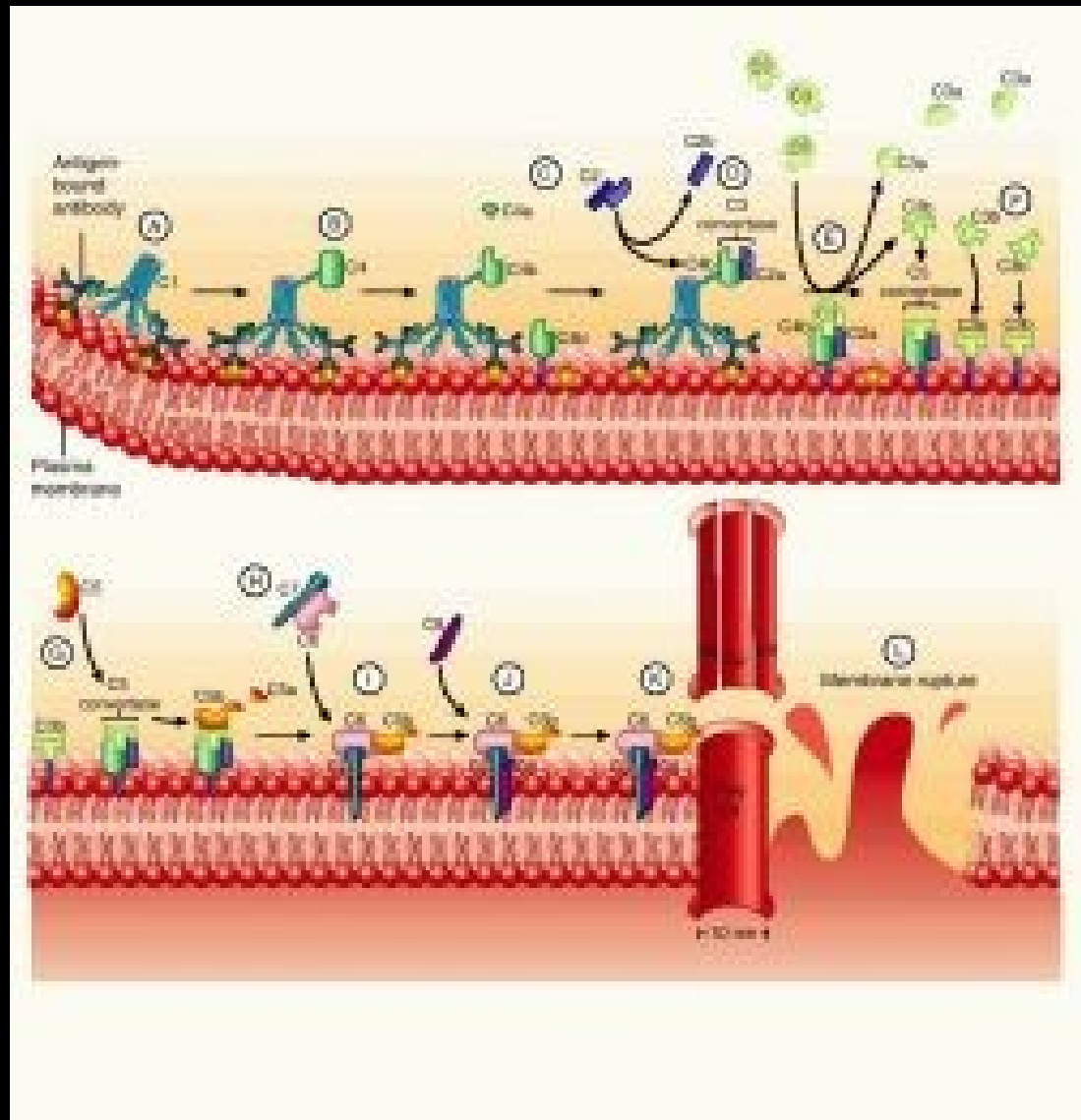
- **1 – 2:** seroconversion
- **3 – 4:** titre elevation
- **5 – 6:** titre decrease



Complement and its properties

The Complement

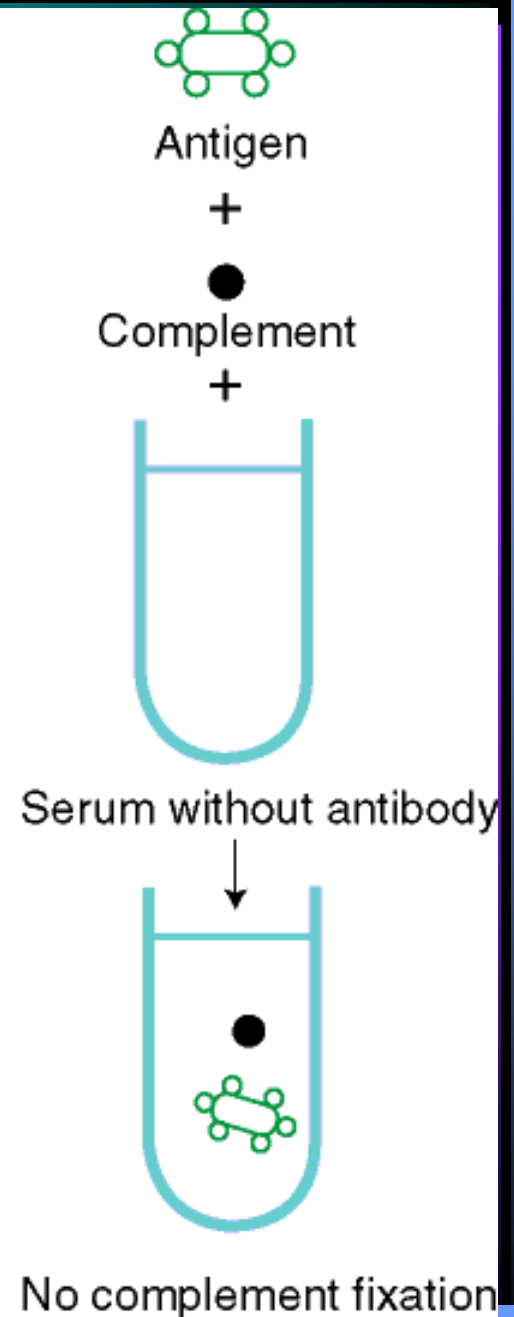
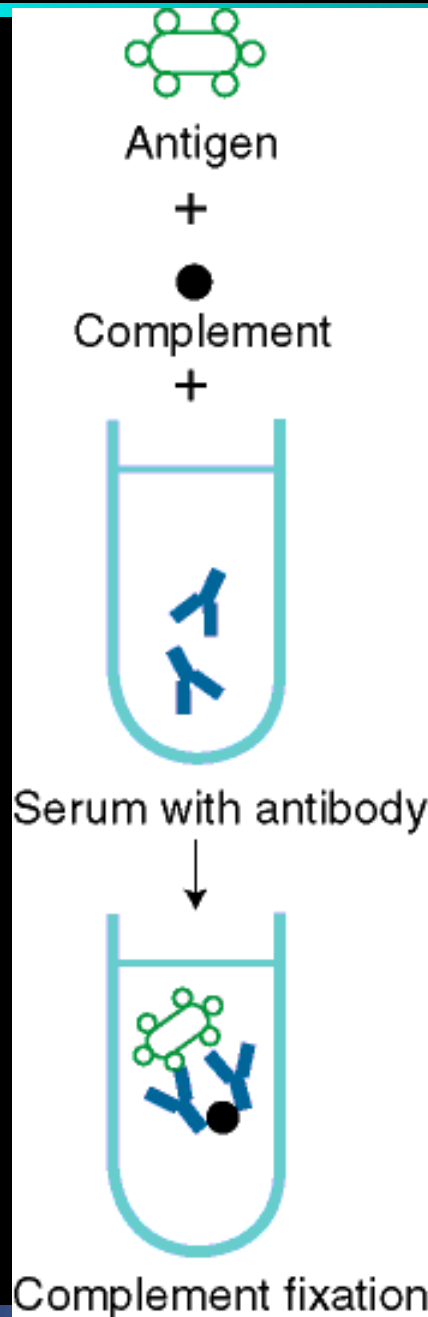
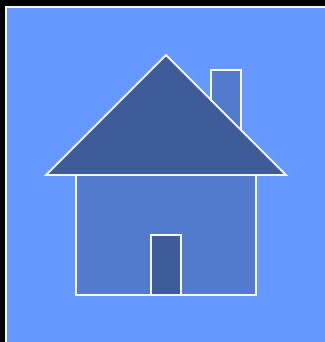
- part of non-specific humoral immunity
- a complex cascade system



Complement-fixing test (CFT)

- Complement = one component of immunity reaction
- For CFT, we use animal (guinea-pig) complement. The patient's complement is inactivated before the reaction
- Complement is **not able to get bound to isolated antigen**
- Complement is **not able to get bound to isolated antibody**
- Complement **is only able to get bound to the COMPLEX antigen – antibody**

Complement and its properties

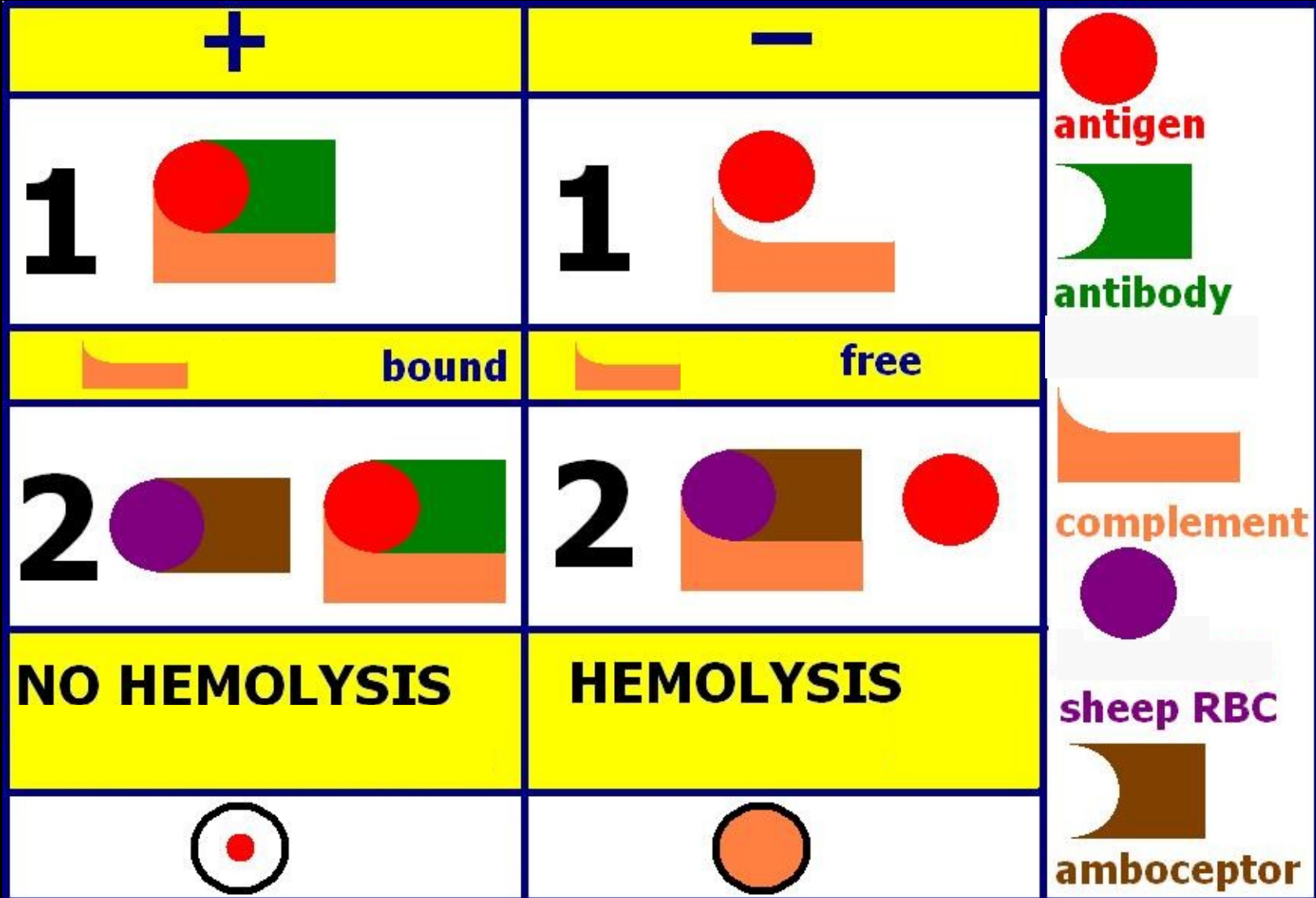


Complement
fixing test
(CFT), its
principle

CFT principle

- Patient serum is mixed with laboratory antigen (*or laboratory animal serum with patients specimen in direct CFT*).
- Complement is added. It binds in positive case (it is only able to bind when a complex Ag-Ig is present)
- In the 2nd phase, we add indicator system (sheep RBCs + amboceptor). In positive reaction indicator system remains intact. In negative reaction the indicator system is haemolysed

CFT – principle



Complement – how it reacts with the indicator system

The haemolysis requires presence of sheep (not rabbit) antibodies, amboceptor and complement. One of components missing or replaced → no haemolysis.

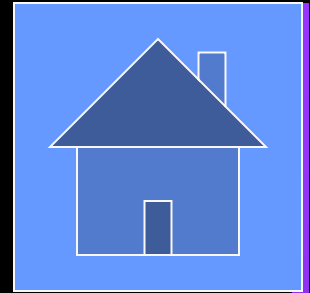
Sheep RBC + amboceptor **without complement** → no haemolysis

Sheep RBC + complement **without amboceptor** → no haemolysis

Rabbit RBC + complement + amboceptor → no hemolysis

Sheep RBC + complement + amboceptor → haemolysis

Use of CFT



- CFT is used for diagnostics of many (mainly viral) pathogens
- CFT, like other serological reactions, may be used for antigen detection or antibody detection
- For simplification, we shall only speak about antibody detection in this practical
- So, we think about a laboratory antigen being mixed with patient's serum (where we search for antibodies)

Trouble shooting in CFT

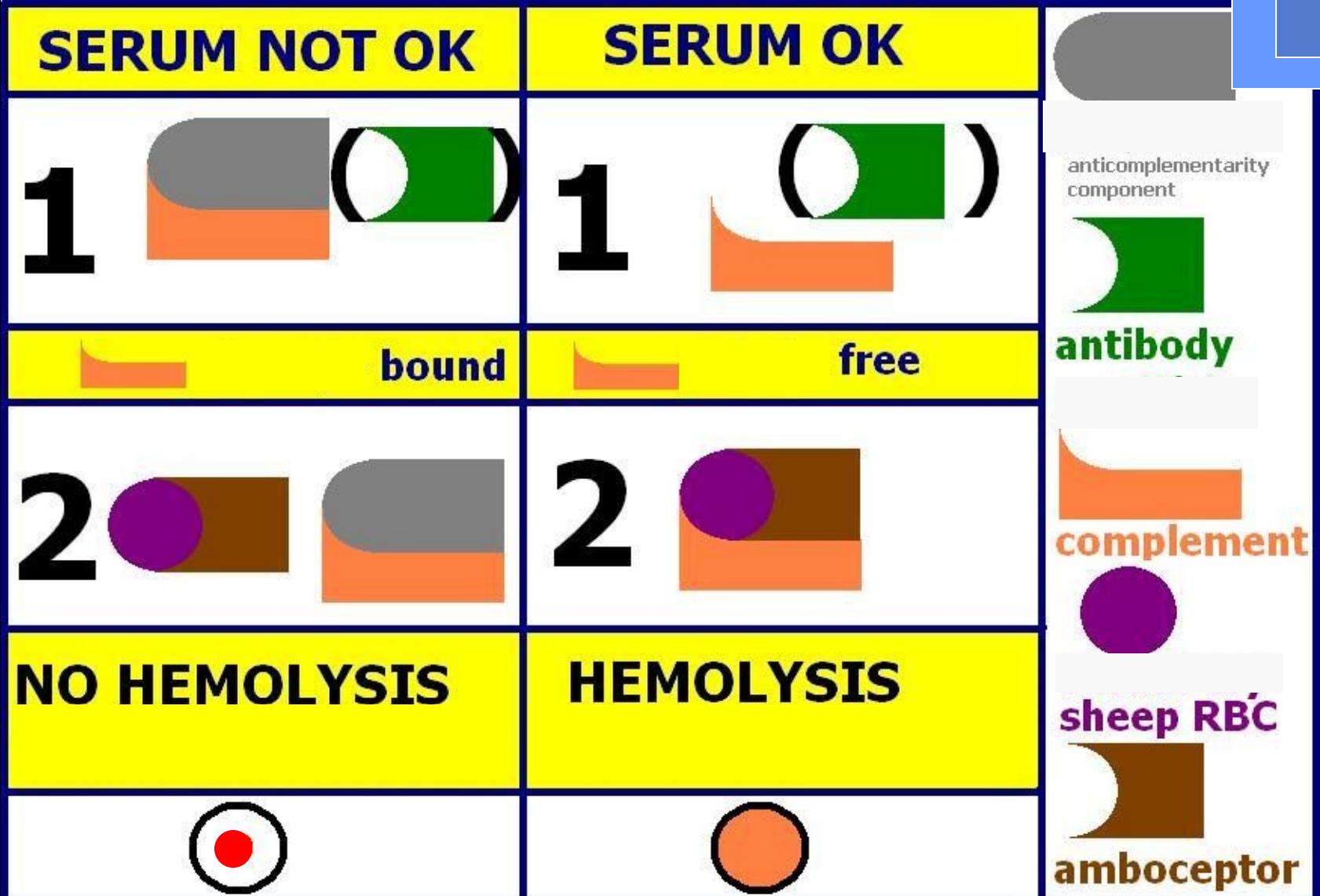
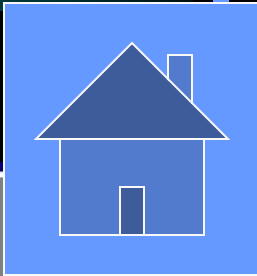
Problems existing in CFT

- Too much complement: false negative results. **What to do? Titrate the complement to assess the proper amount**
- Something in serum binding the complement itself (anticomplementarity component): false positive results. **What to do? Perform anticomplementarity test – like normal course of CFT, but without antigen** (*A situation like a homeless man sweeping the plant globules from the bench, even when the boy did not come into the park because he was ill*)

Titration of complement

- For the reaction, we need an amount of guinea-pig complement that is neither too small nor too big
- That is why we test, **what amount of complement is just able to perform haemolysis of a specified amount of red blood cells with amboceptor**
- *Too big amount of complement → false negativity (too many plant globules → some of them remain for niece&boy-friend)*

Anticomplementarity test



Examples of CFT use in practice

Clinical situation A

- A patient with long term respiratory problems, a few clinical signs, the most probable diagnosis: **atypic pneumonia**
- Atypic pneumonia may be caused by many **respiratory viruses**, but also several **bacteria** (*Mycoplasma*, *Chlamydia*)
- Eventual **mycoplasmal/chlamydial etiology** would mean effect of antibiotics. In viral etiology antibiotics would have no effect

Respiratory pathogens

- The whole seropanel belongs to one patient.
- We have **six respiratory pathogens**, each in **two rows** (acute specimen, convalescent specimen).
- **First column = the anticomplementarity test**
- Then we have **seven dilutions of sera**, i. e. dilution 1 : 5 in 2nd column, 1 : 10 in 3rd etc., with coefficient two. Besides viruses, a bacterium *Mycoplasma pneumoniae* is in the panel, too (difficult culture)

Clinical situation B

- We have three patients with suspicion for tick-borne encephalitis, all of them with neurologic symptoms and anamnesis of being bitten by a tick
- Tick borne encephalitis is a disease quite common in central Europe. Although it has worse course in adults (mostly seniors), people tend to vaccinate rather their childrens and not their parents.



Tick-borne encefalitis

- We test antibodies again, now against tick-borne encefalitis.
 - positive control in the first row
 - in 2nd and 3rd row the first patient
 - in 4th and 5th row the second patient
 - in 6th and 7th row the third patient
- Each patient has two rows (acute serum and the convalescent one)
- In the first column, we have anticomplementarity tests again, and then sera dilutions, starting from 1 : 4 (continued: 1 : 8, 1 : 16, 1 : 32, 1 : 64 etc.)

Clinical situation C

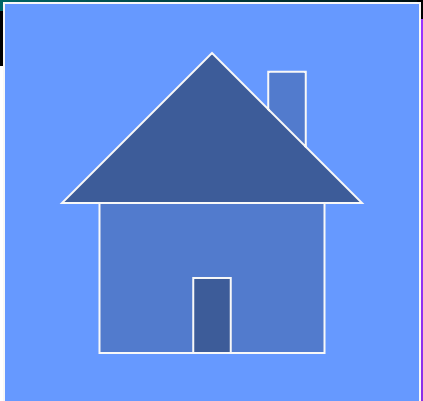
- We have several patients that should be screened for presence of antibodies against toxoplasmosis (*Toxoplasma gondii* is a tissue parasite, cat is the definitive host)
- Seronegativity means that the person never met the infection*. Seropositivity should be studied in more details (one more sampling, eventually ELISA reaction for immunoglobulin class assessment)



**Or the infection is so fresh that the antibodies had no time to be created.*


Toxoplasmosis

- The seropanel belongs to a positive control (1st row) and three patients (2nd to 7th row)
- We search for antibodies against toxoplasmosis.
- **There are anticomplementarity tests in the first column,** and then dilution by geometric row starting from 1 : 8.
- Each patient has only one row (we do not follow titre dynamics)

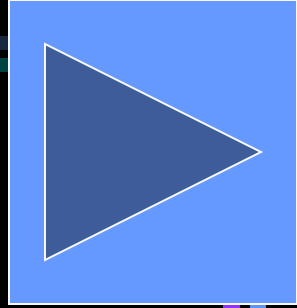


<http://www.presse.uni-wuppertal.de/archiv/output/okt98>

Tick borne encephalitis virus

- **Tick borne encephalitis** often infects children, serious symptoms are rather typical for adults. Despite that adults rarely let themselves **vaccinated**. In the first phase it has flu-like symptoms, in the second meningeal or cerebral symptoms. Letality of infection is 1–5 %. 
- It is a typical **arbovirus** (=arthropode borne virus), rodents are source
- **Diagnostics** is mostly indirect.

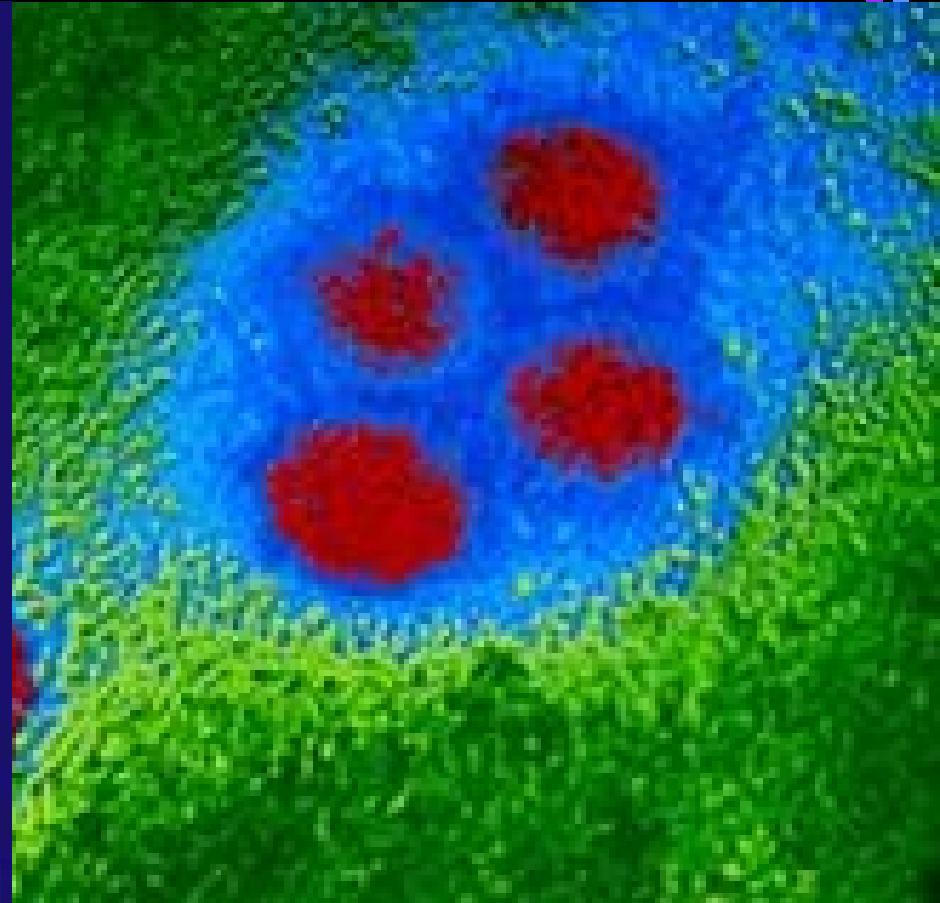
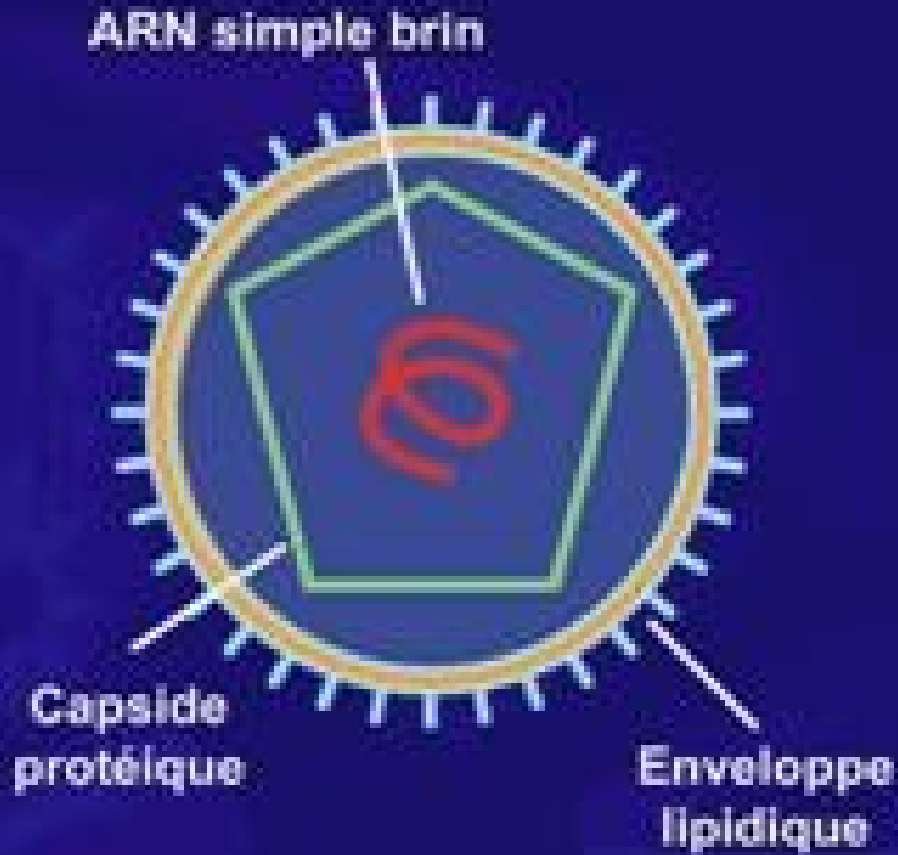
More flaviviral encephalites and fevers



- Besides Central-European **tick borne encephalitis** we have more tick borne encephalites. Russian spring-summer encephalitis, is another subtype to the Central-European, less related is the scotish „louping ill“ and Omsk haemorrhagic fever.

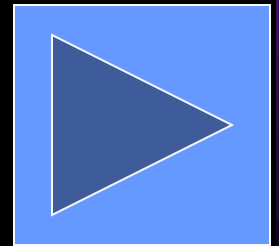
- Also there exist **Japanese encephalitis**, transmitted by mosquitoes of genus *Culex*. Related is also **West Nile fever**, also mosquito transmitted. It is likely that is is present even in Czechia around Lanžhot

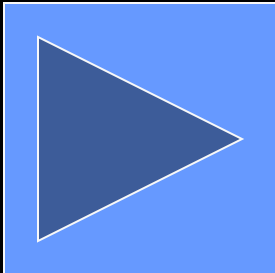
Virus of tick borne encephalitis



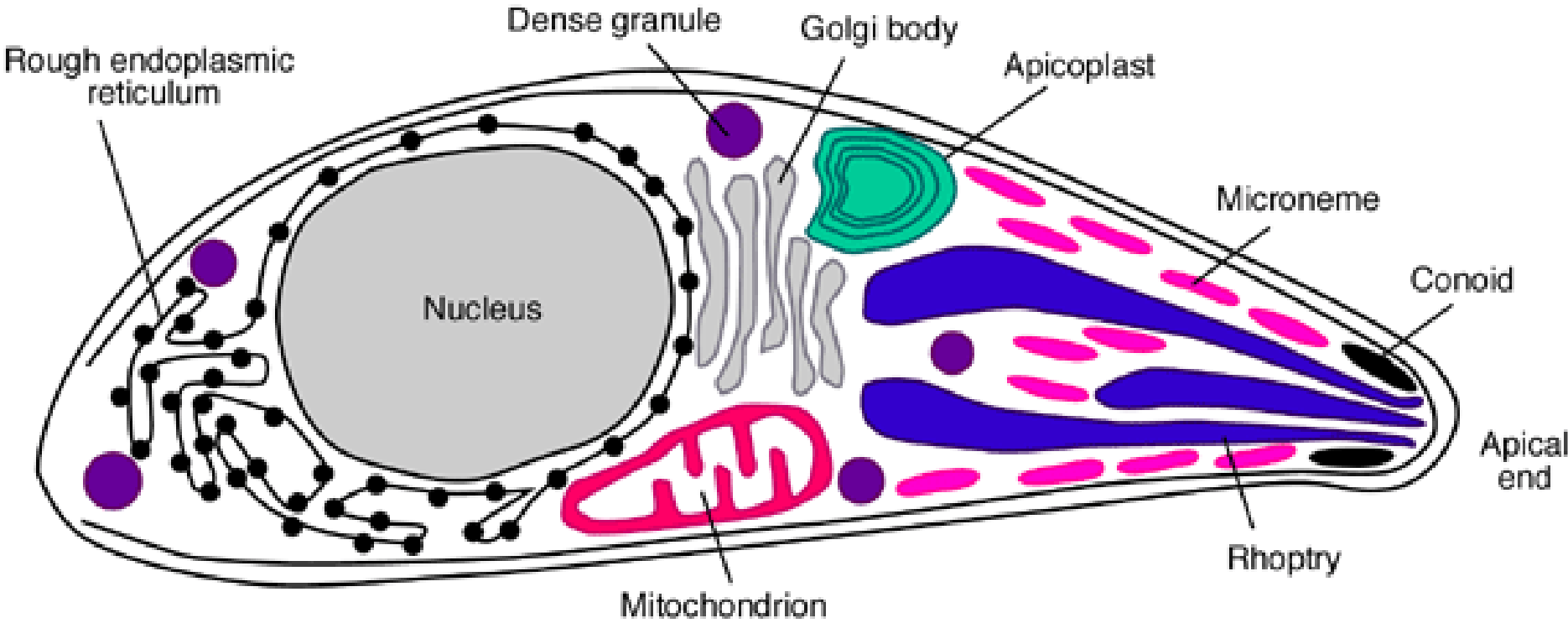
Toxoplasma gondii

- It is a protozoon; cats are its source, but people having dogs are in higher risk (dogs use to have cat faeces in their fur)
- Majority of infections in immunocompetent persons is asymptomatic, or only temporarily enlarged lymphonodes are observed.
- Ocular form is dangerous
- Infection of foetus is dangerous, too, especially in 1st trimester



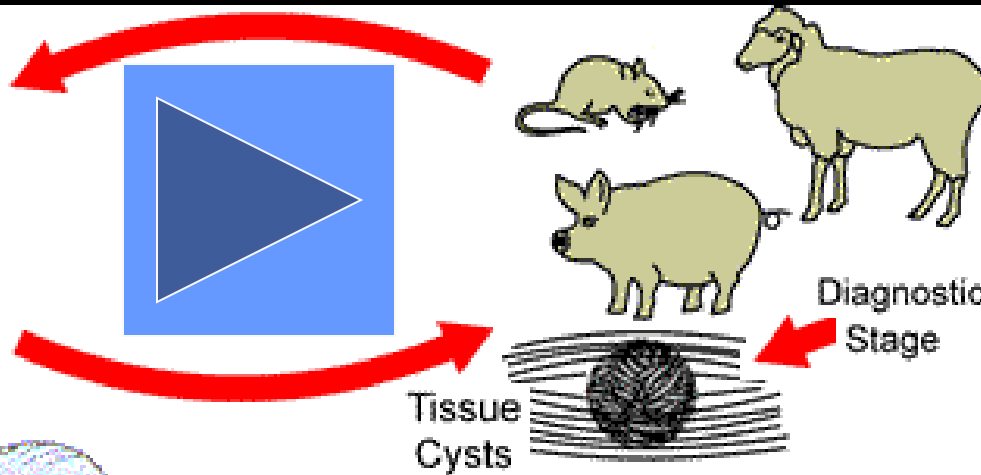


Toxoplasma gondii

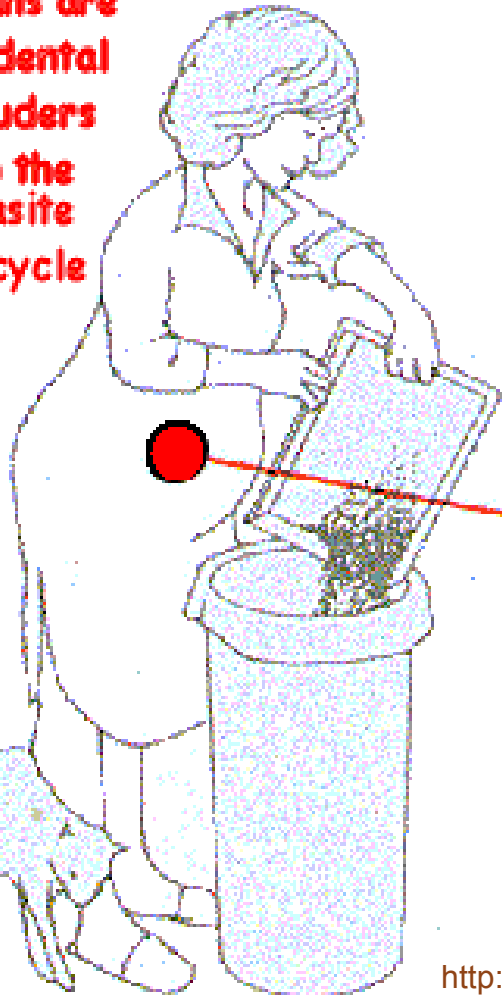


Ultrastructure of a *Toxoplasma gondii* tachyzoite
Expert Reviews in Molecular Medicine ©2001 Cambridge University Press

Definitive Host

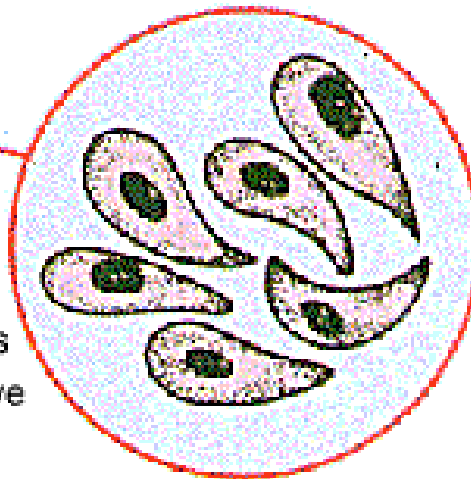


Humans are accidental intruders into the parasite life cycle



Both oocysts and tissue cysts transform into tachyzoites shortly after ingestion. Tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites. If a pregnant woman becomes infected, tachyzoites can infect the fetus via the bloodstream.

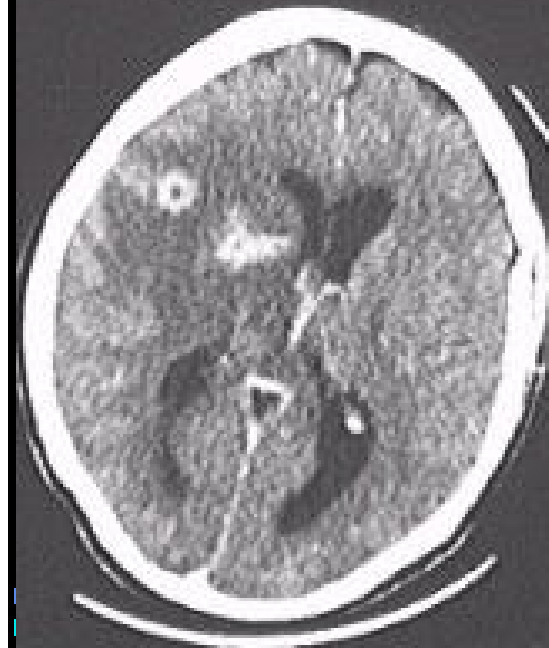
Fecal Oocysts = Infective Stage

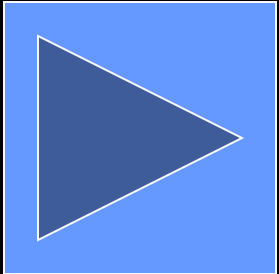


Toxoplasma life cycle

Down:
Toxoplasma cyst in brain

http://www.antoranz.net/CURIO/SA/ZBIOR3/C0311/03-QZC08043-3_Toxoplasma.jpg

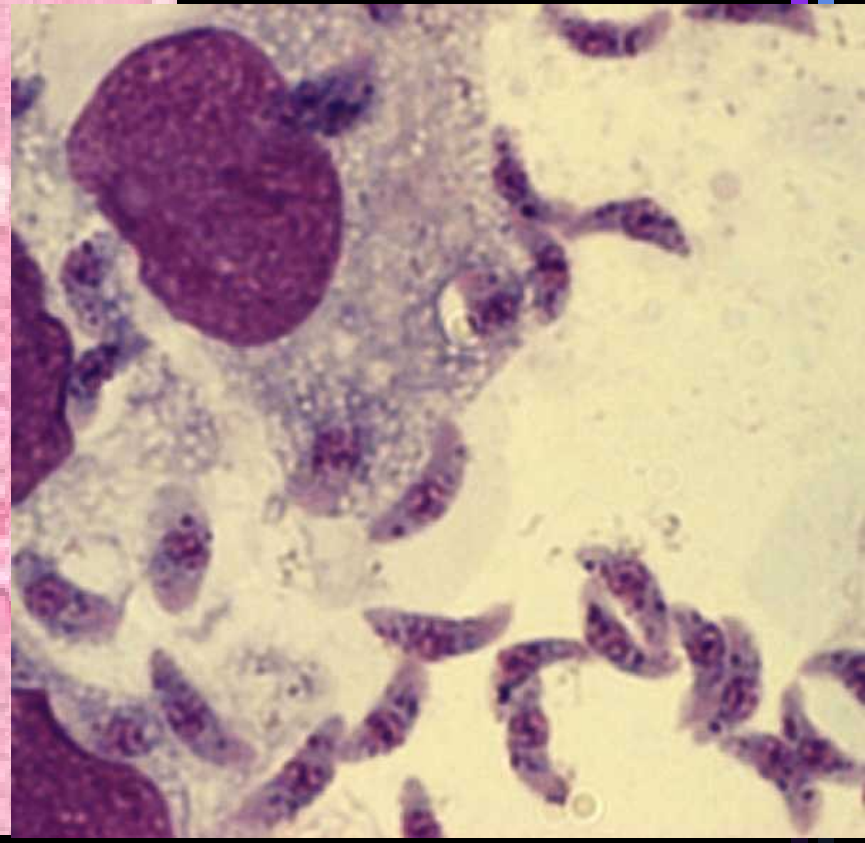
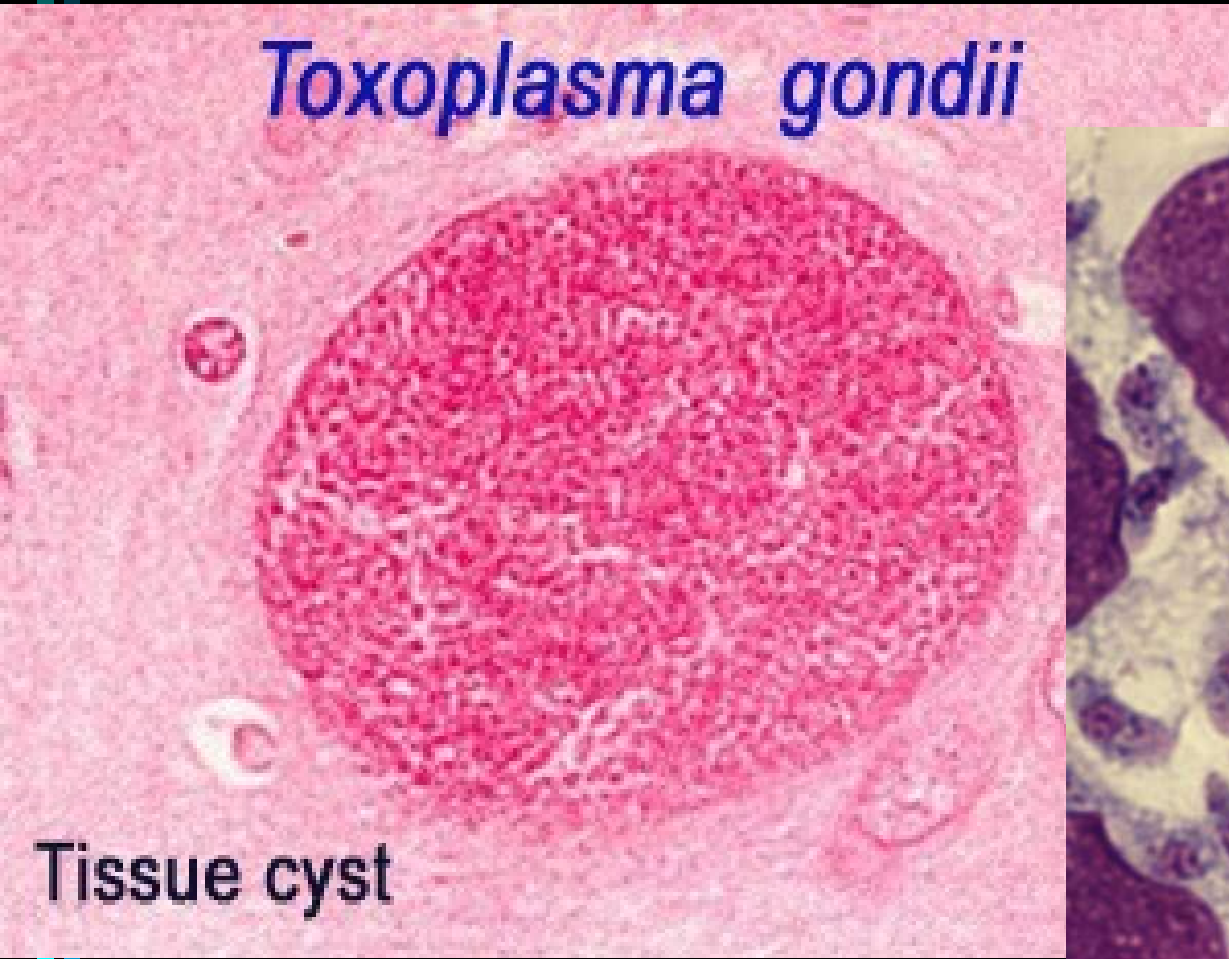




http://webdb.dmsc.moph.gov.th/ifc_nih/applications/pics/Toxoplasma.jpg

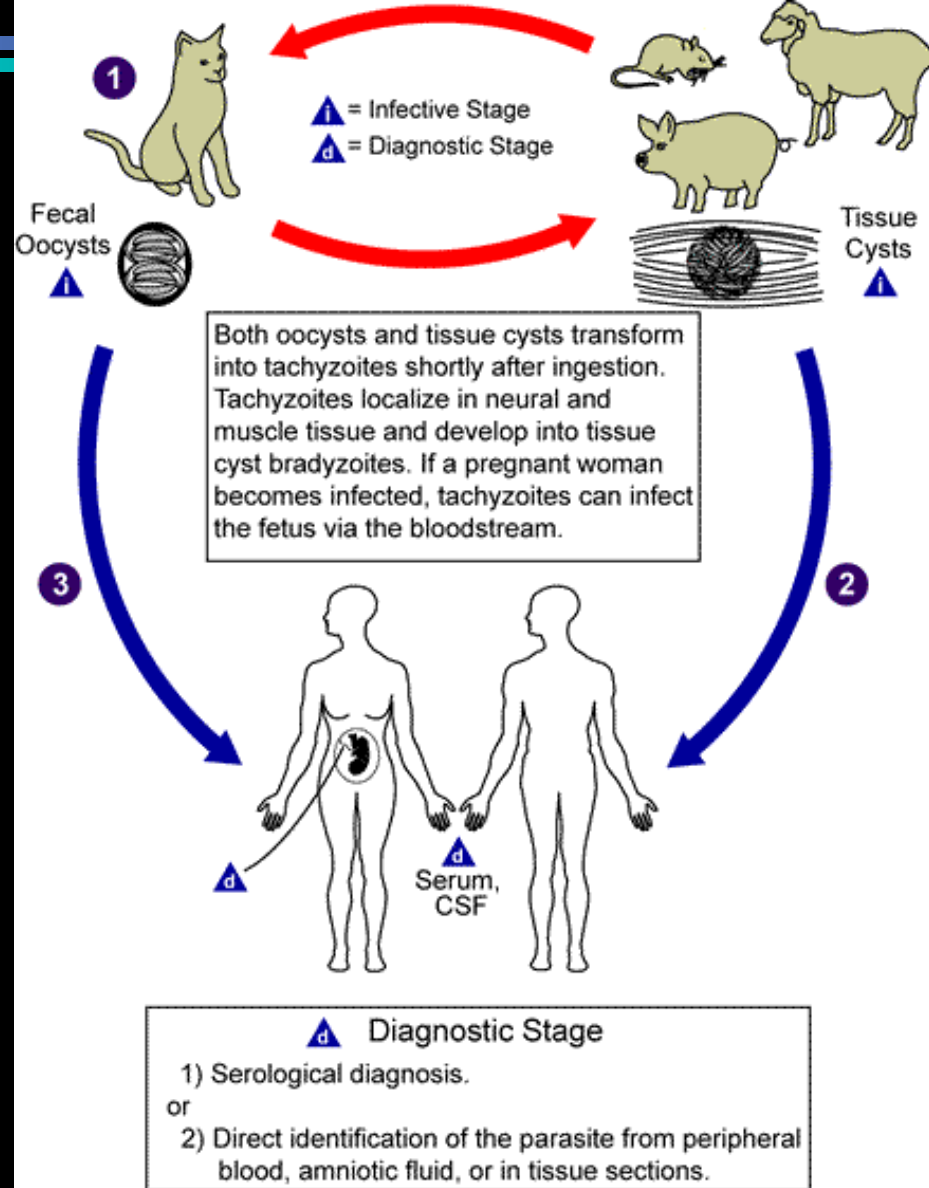
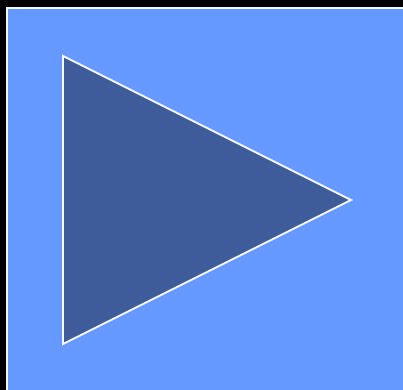
Toxoplasma gondii

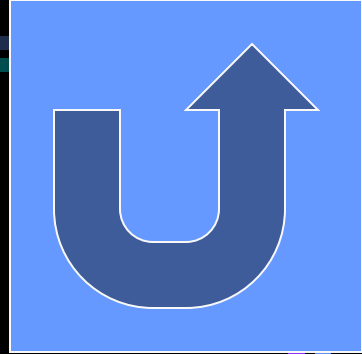
<http://www.smittskyddsinstitutet.se/upload/Analyser/ToxoplasmaSB.jpg>



Tissue cyst

Toxoplasma – life cycle



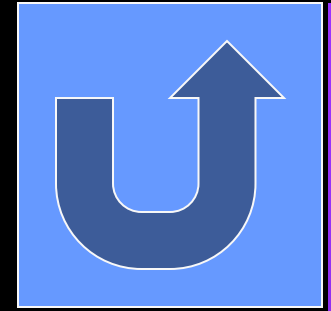


In some
persons,
toxoplasma
retinitis
may
occur...

Toxoplasma
Retinitis



Lortality and mortality



- **Lortality** is the ratio between the persons dying for the disease and the total of infected persons
- **Mortality**, on the other hand, is the average number of persons dying for a disease (usually counted per 100 000 inhabitants and one year)