Searching for microbes
Part XIII.
Virology – Part Two

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Content of this slideshow

Viruses of influenza

More respiratory viruses

Mycoplasmas

Virus of tick-borne encephalitis

Some viral properties important for diagnostics

Survey of virological diagnostics

Microscopy in virology. Viral isolation

Indirect diagnostics of viruses

Story

- Mr. Lungman was already a week in a hospital because of long term respiratory problems. A group of medical students started to examinate him, but they found no pathological finding.
- But then a student, Miss Diligentous spoke: „Patient has a dry cough, physical examination showed nothing. What about an atypical pneumonia?“ The teacher smiled: Excellent, I am glad that someone found it!

Atypical pneumoniae

- have a slow oncome, rather non-productive cough, and physical symptomatology is often absent.
- Agents of classical pneumoniae (e. g. *Streptococcus pneumoniae*) are not found here, usually it is one of respiratory viruses. Nevertheless, it could be an atypical bacterium, e. g. *Mycoplasma pneumoniae*, or *Chlamydophila pneumoniae*.
- In case of bacterial agents, antibiotic therapy (doxycyklin, macrolids) is possible

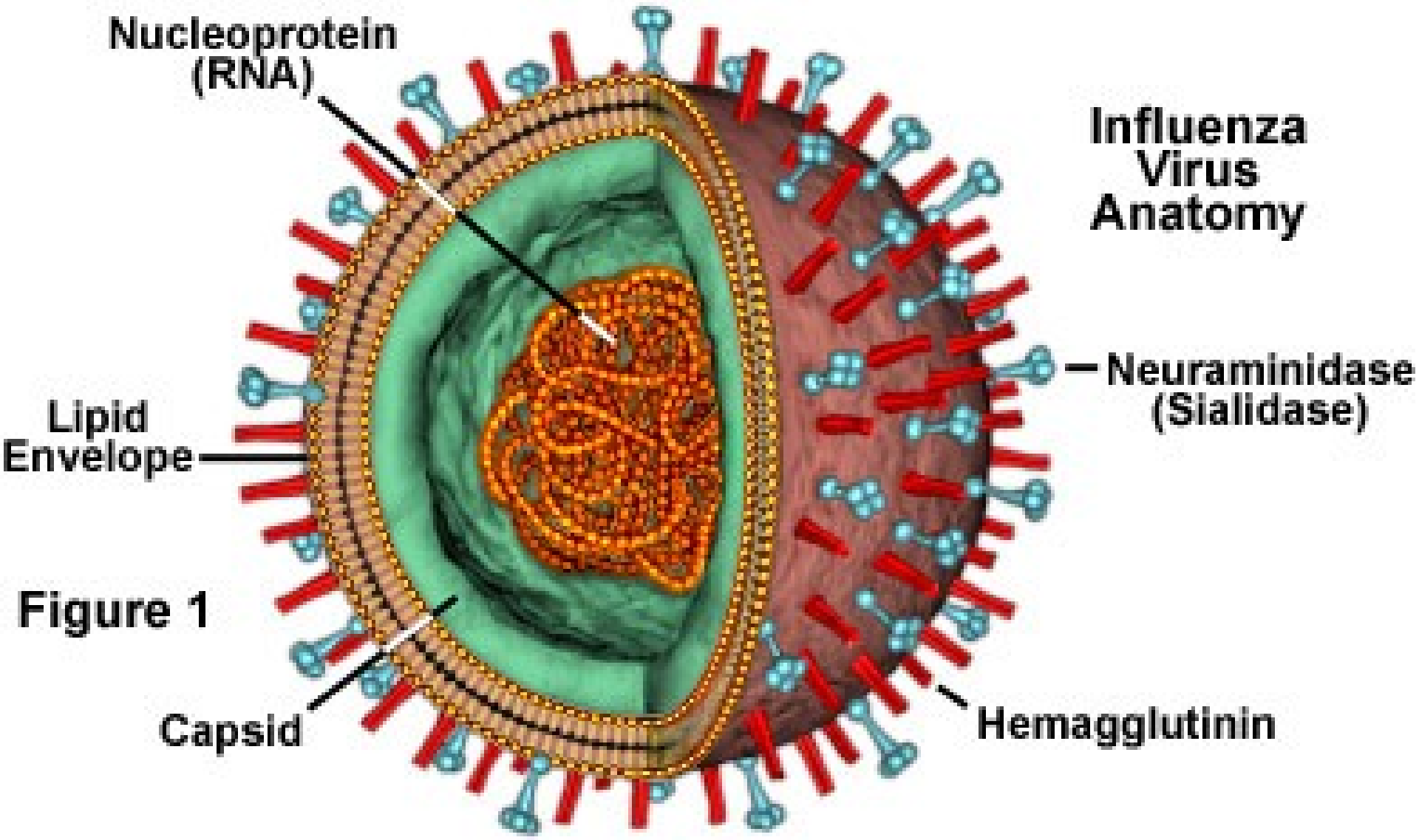
Viruses of influenza

Influenza (flu)

- It is a disease of the **whole body**, but mostly **lower respiratory ways**. Neither rhinitis, nor sore throat belongs to typical course. Rather dry cough, high fever, myalgia, accute oncome. Sometimes also intestinal problem may occur.
- Influenza is often **dangerous** in immunocomprmissed persons (mostly prolonged), pregnant, in elderly. On the orher hand, some subtypes are rather dangerous for young people with very good imunity. This is because of „**cytokin storms**" (the problems are related with imunity overreaction)
- Influenza A, B and C exist; majority of epidemics is caused by **virus of influenza A**

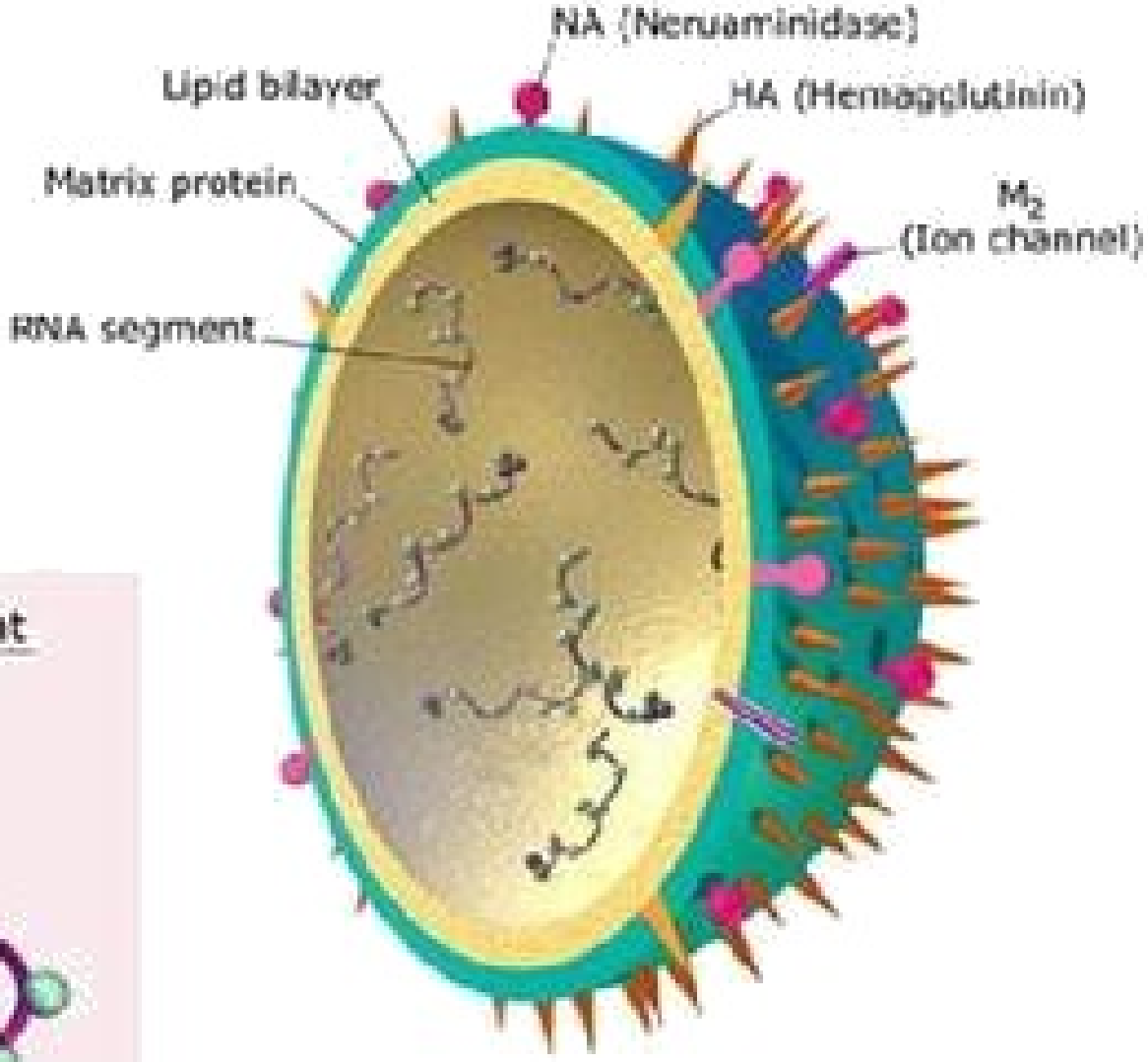
Influenzavirus A

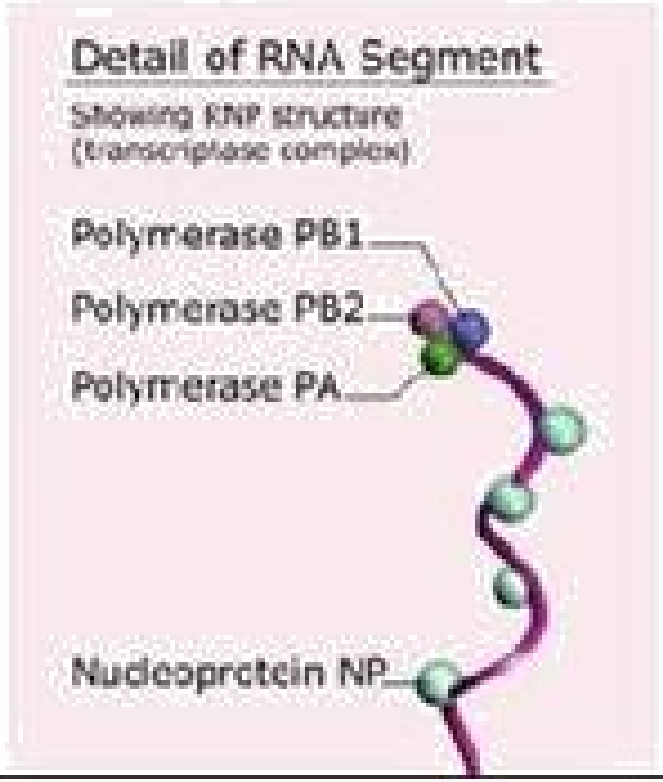
http://micro.magnet.fsu.edu/cells/viruses/influenzavirus.html



Influenzavirus A – antigens

- Among characteristics of influenzavirus, the most important is antigenic variability. In influenzavirus, we have 15 subtypes according to haemaglutinin antigen (H) and 9 subtypes according to neuraminidase (N).
- A light **antigenic drift** is common – tiny changes of antigenic structures
- An **antigenic exchange – shift** means, that a new subtype is found, with a new H, eventually N. It can be genetically transformed, with formation of a hybrid.





Influenza pandemics

- As viruses change, sometimes a **new variant** occurs and nobody is protected against it. Such a virus is then able to produce outbreaks, epidemics or even **pandemics** in large areas. Of course, it is never possible to predict the course of them.
- Viruses able to perform an outbreak in human population should have not only **elevated virulence**, but also the ability to be transmitted **person-to-person**. Bird viruses rarelly have such ability. Hogs usually serve as „mixing jar". So virulence factors from birds usually become dangerous after **reassortment or recombination with parts of mammal viruses**.

Influenza pandemics 2

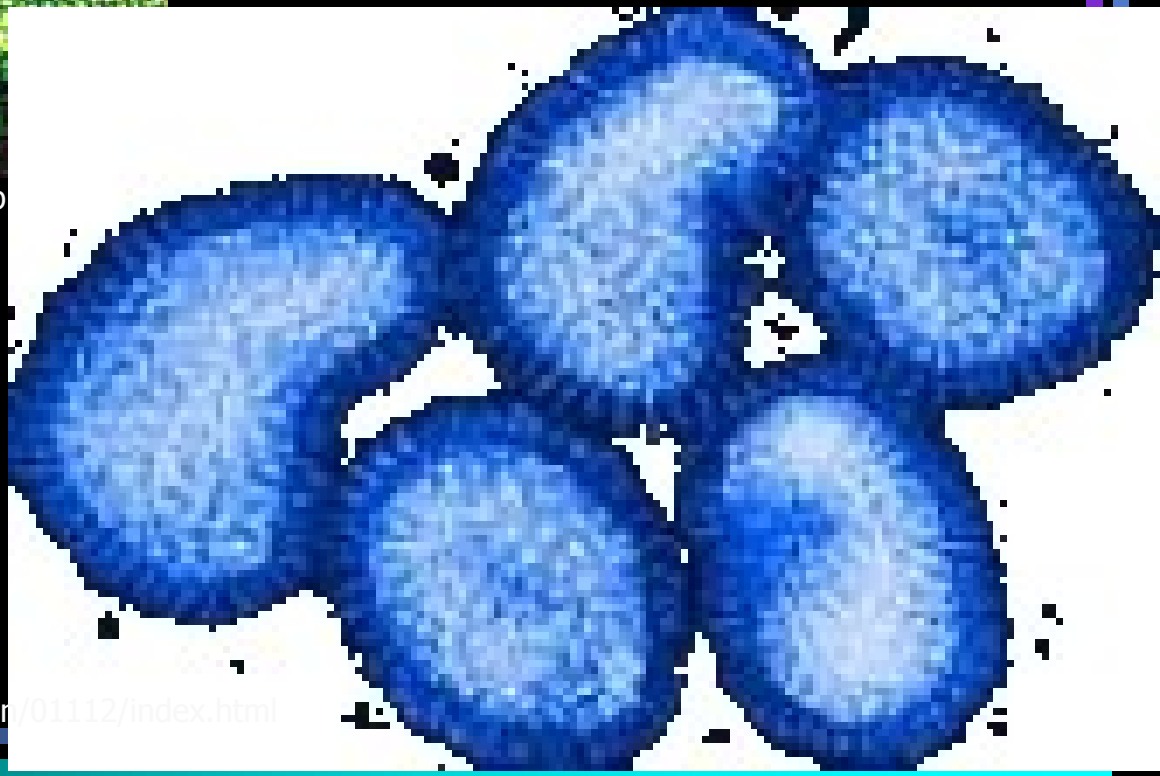
- During World War One many people died because of so called **Spanish influenza**
- During following decades, there were many relatively smaller epidemiae (Hong-Kong flu, Singapore flu)
- The **recent epidemics** is caused by a virus belonging to group A:H1N1. The mere term „A:H1N1" does not mean anything new, but the detail structure is special, the virus contains parts that are human, avian and swine origin

Influenza – prevention, profylaxis, treatment

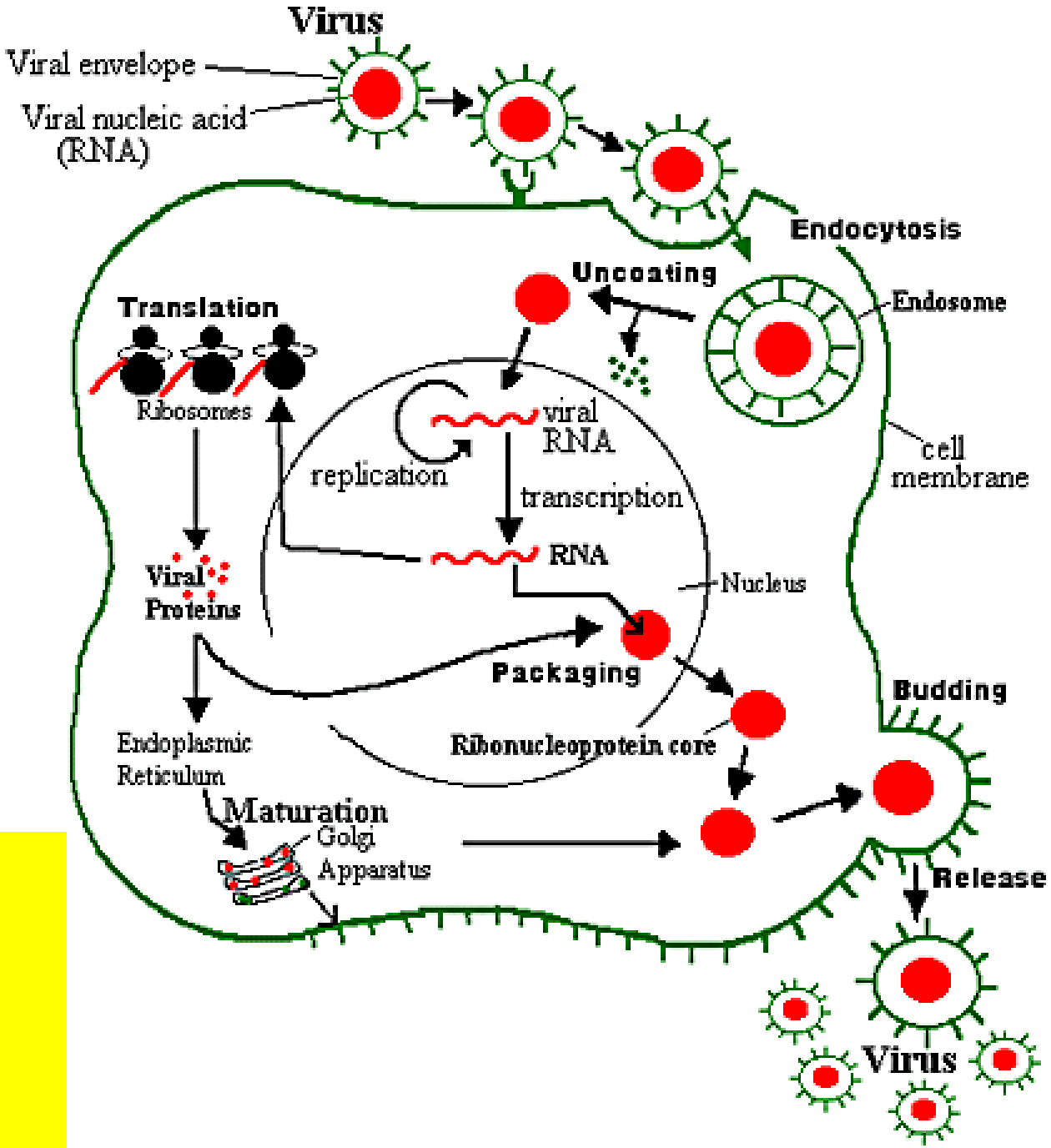
- **Prevention** possible by vaccination, recommended mostly to diseased persons. It protects only agains subtypes actually present in population, not againts new viral subtypes
- For **profylaxis and treatment,** we can use some antivirotics: inhibitors of protein M_2 (amantadin and rimantadin, some strains became resistant already), and inhibitors of neuraminidase (zanamivir and oseltamivir – factory names TAMIFLU and RELENZA).
- Antivirotics should be used for relevant reasons only. Using them „preventively" because of panic might lead to **development to resistance**.

Influenzavirus

www.ontariogenomics.ca/education/episode6.asp



http://www.bio-pro.de/en/region/rhein/magazin/01112/index.html



Influenzavirus – life cycle

In flew Enza ☺

From book „A practical guide to clinical bacteriology“, Pattison JR et al., Wiley, London 1995

More respiratory viruses

Parainfluenzaviruses

- They are paramyxoviruses, related with mumps virus and less related with measles
- Unlike the true flu they often cause **cathars of upper respiratory ways**. Nevertheless, flu-like cough may be too, but mustly (especially in adults) without fever.
- **Diagnostics:** CFT, HIT, ELISA; there are some cross-reactions. A direct diagnostics in nasopharynx using isolation on tissue culures possible, too.

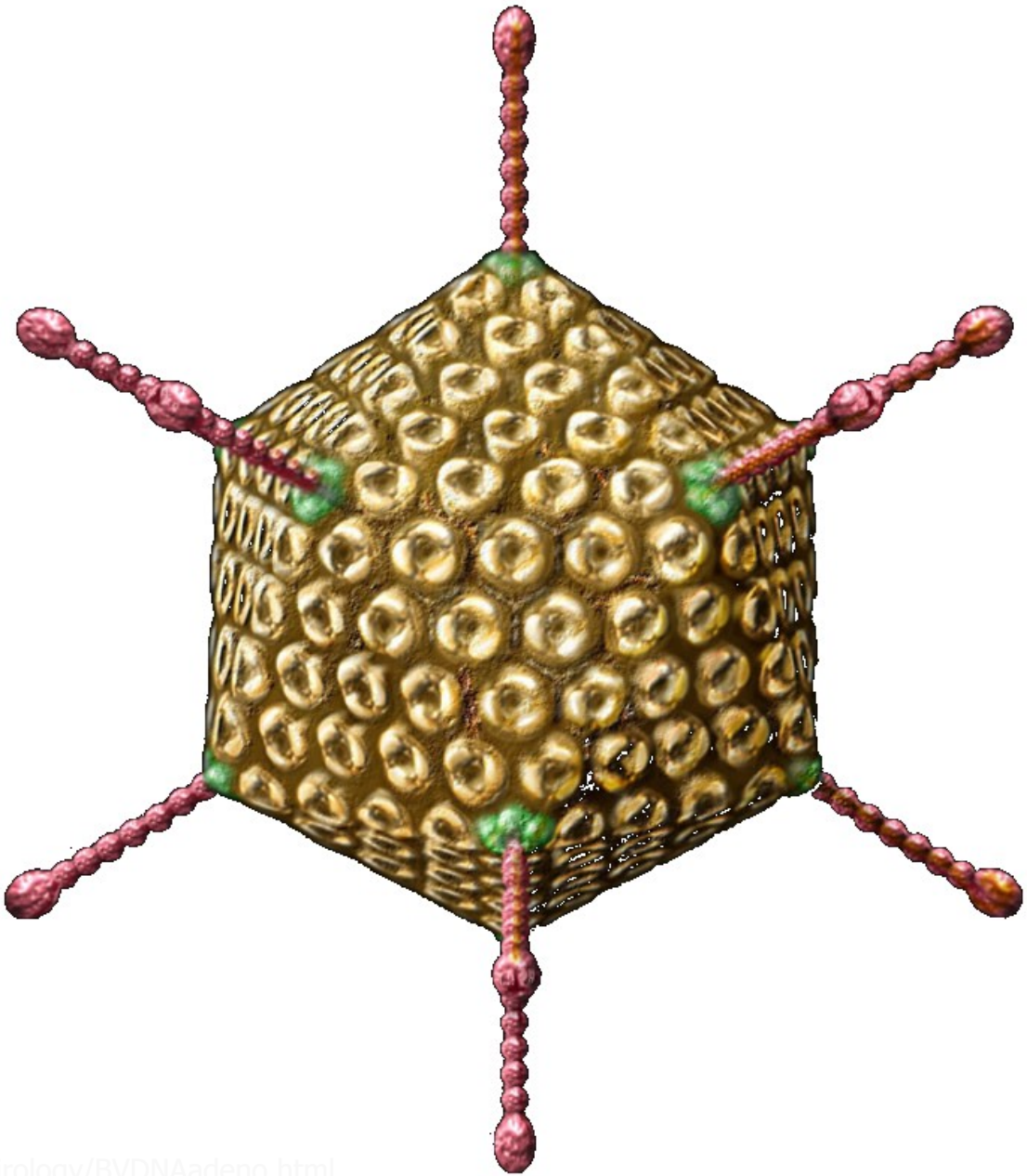
RS virus (respiratory syncytia virus, pneumovirus)

- Related (not very closely) to parainfluenza
- RS-virus is an **important pathogen of lower respiratory ways in first halfth of year**
- As the name says, they cause **confluence of infected cells** (syncytia)
- **Diagnostics** – ELISA, direct dg. – tissue cultures

Adenoviruses – Uncoated DNA viruses

- First isolated 1953 from an adenoid vegetation
- They are human, animal and bird viruses
- They are medium sized (80 nm), uncoated, with cubic symetry of capsid. They have shape of a perfectly regular icosaedre. The capsid is composed of 240 hexons a 12 top pentons.
- We know 47 serotypes of adenoviruses, that can be pathogenous for humans. They coud differ in symptomas and diagnostic.

Adenoviru

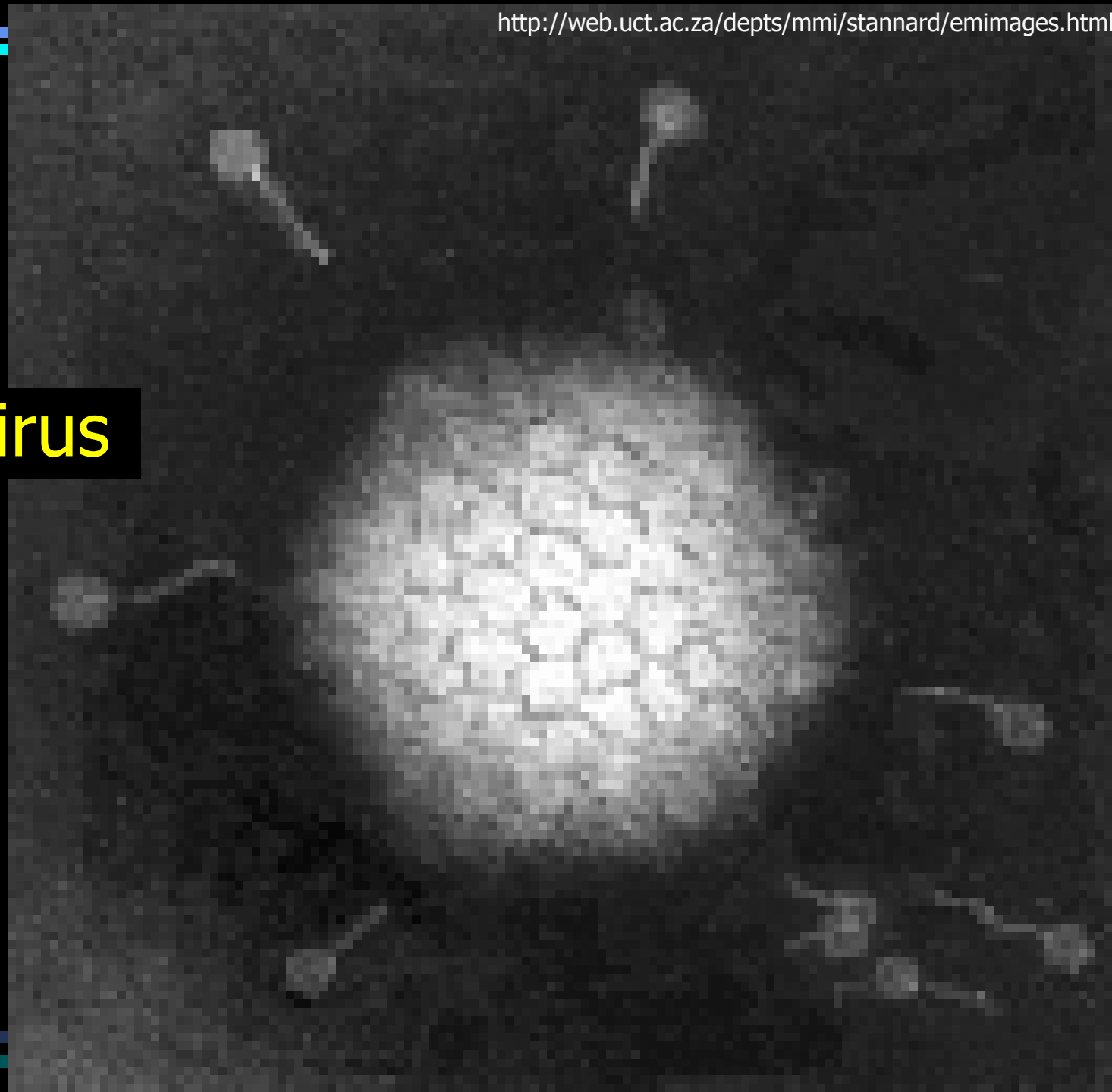


http://www.tulane.edu/~dmsander/Big_Virology/BVDNAadeno.html

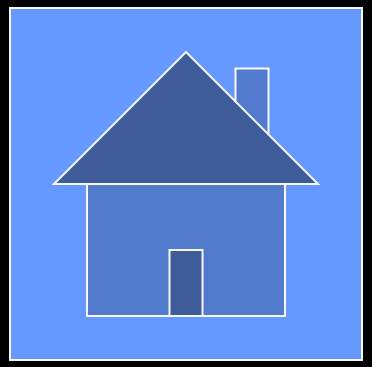
Human adenoviruses

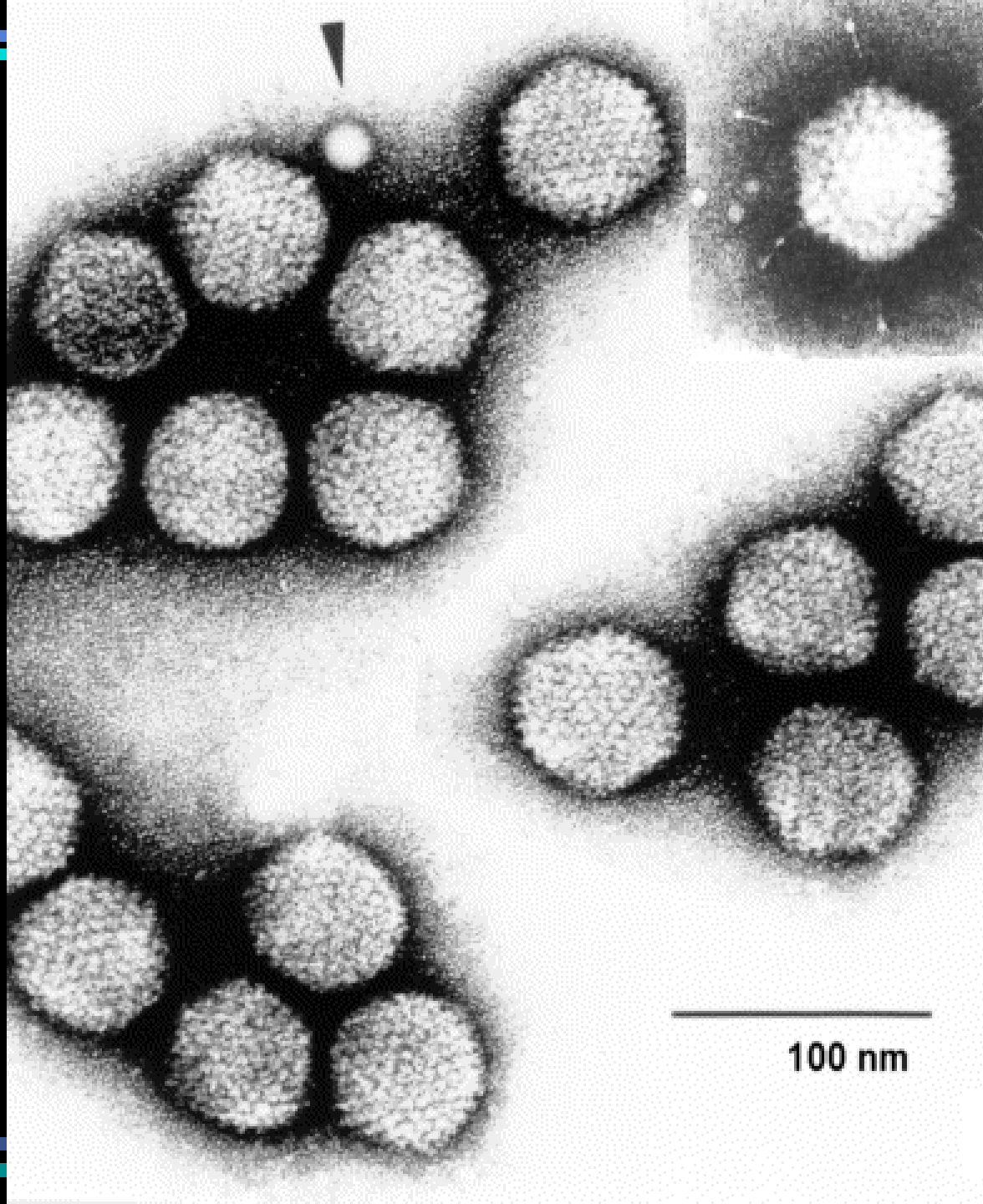
- They may cause **common cold, pharyngitis, conjunctivitis** (light to serious)
- Types 40 and 41 (different also by being unculturable) cause **diarrhoea in babies**
- One type may cause **inflamation of urinary bladder with bleeding**
- **Diagnostics** is culture (tissue cultures) and serological (complementfixing test)
- **Target therapy** is impossible

Adenovirus



Adenoviruses





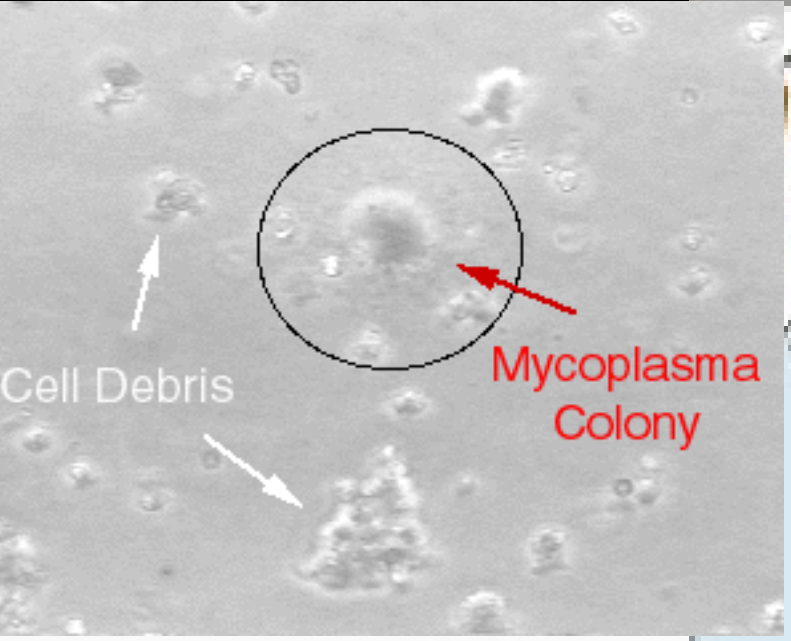
http://www.tulane.edu/~dmsander/Big_Virology/BVDNAadeno.html

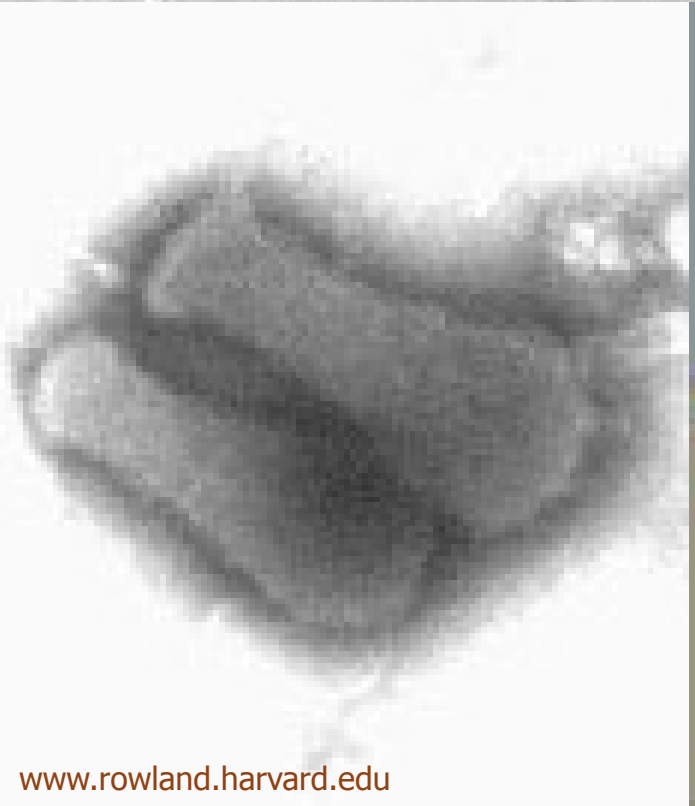
Mycoplasmas


Mycoplasmas
http://www.unmc.edu/dept/biochemistry

Mycoplasmas

- strange group of bacteria – Mollicutes – „the ones with soft skin"
- **they have no cell wall**
- their shape might be oval, round or filamentous
- in humans, genera ***Mycoplasma*** and ***Ureaplasma*** are important
- smallest organisms with no need for an alien cells
- several times smaller than common bacteria





www.rowland.harvard.edu

www.invivogen.comv

Mycoplasma pneumoniae

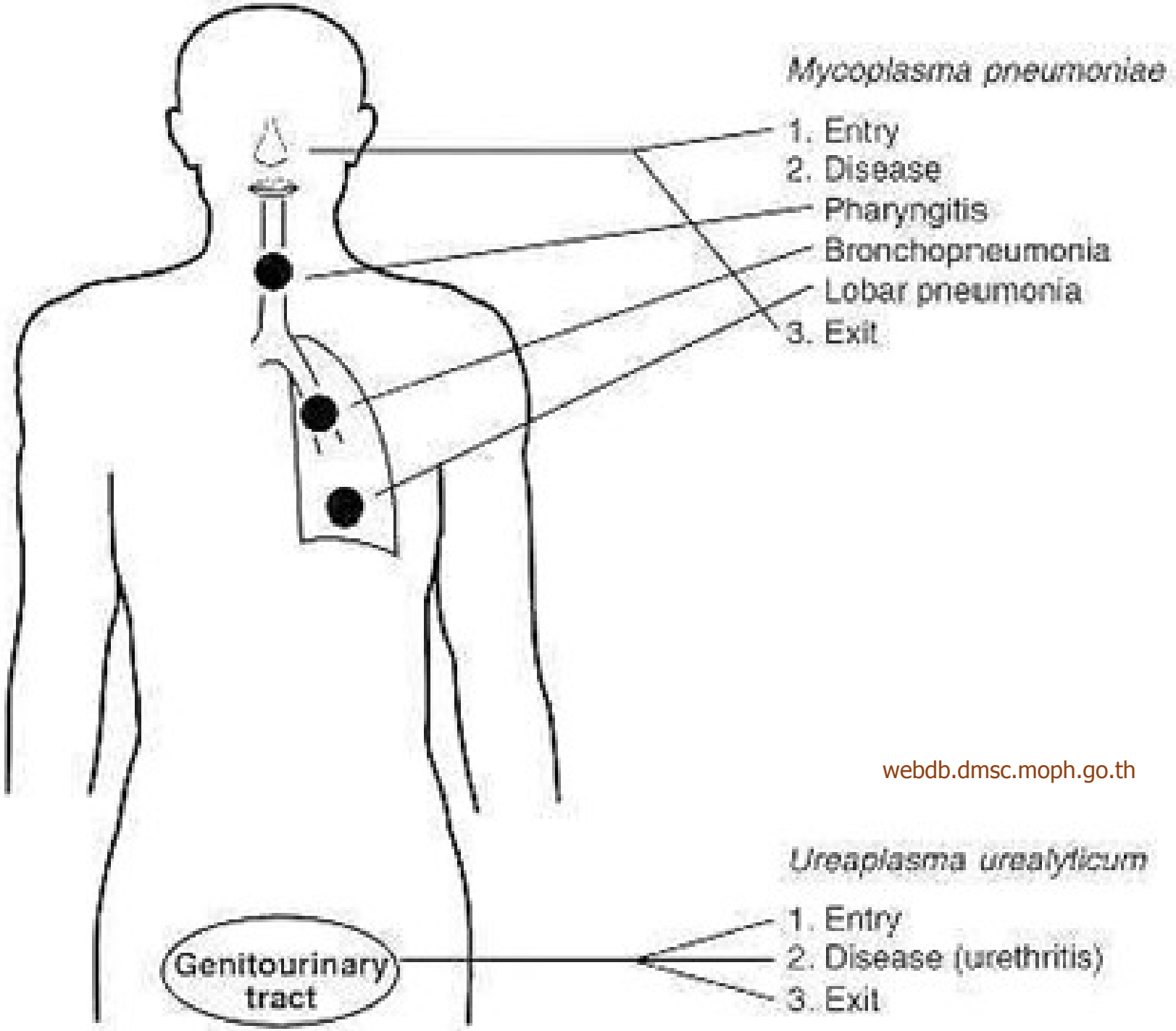
- causative agent of atypic pneumoniae
- sometimes extrapulmonar complications (hearth, nerves etc.)
- on the other hand, often only common-cold-like or asymptomatical
- transmission by air

Mycoplasma hominis, Ureaplasma urealyticum

- important agents of sexually transmitted diseases
- inflammation of vagina and urethra

Other urogenital mycoplasmas:

- *Mycoplasma genitalium* also sexual organs, uclear importance
- *Mycoplasma penetrans* as an opportune infection in AIDS patients


Mycoplasma pneumoniae
1. Entry
2. Disease
Pharyngitis
Bronchopneumonia
Lobar pneumonia
3. Exit
webdb.dmsc.moph.go.th
Ureaplasma urealyticum
1. Entry
2. Disease (urethritis)
3. Exit
Genitourinary tract

Mycoplasmas

webdb.dmsc.moph.go.th

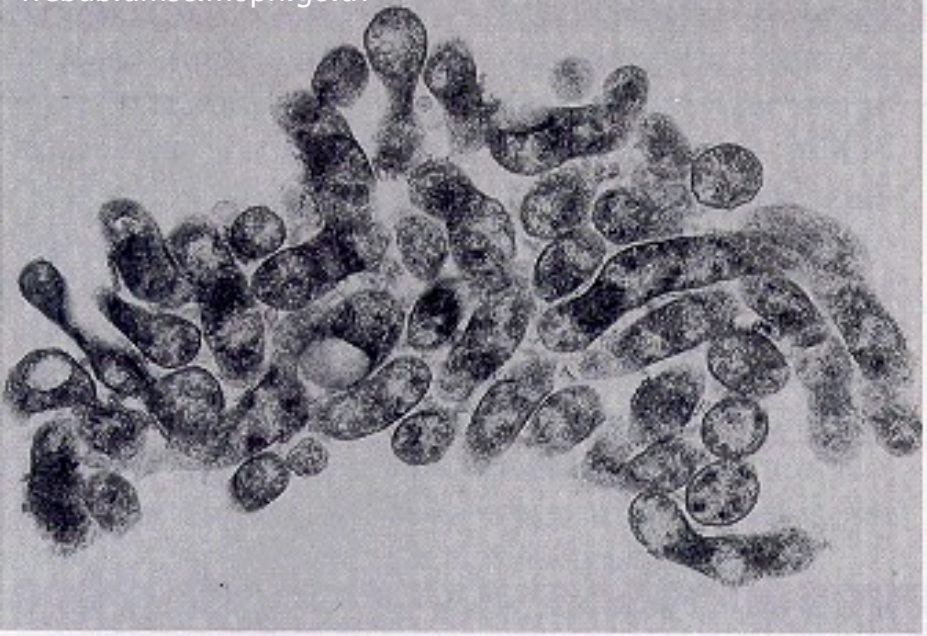
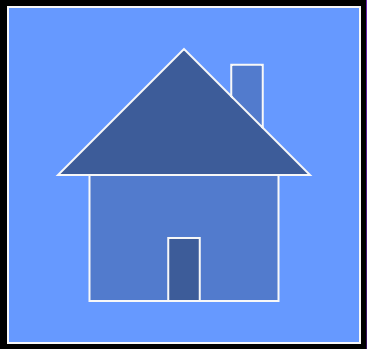


Fig. 17-83 *Mycoplasma.* Electron micrograph of *Mycoplasma pneumoniae*. The cell lacks a cell wall and is bounded by a cytoplasmic membrane that has a trilaminar structure.

Mycoplasma: diagnostics and treatment

- Culture on acelullar, nevertheless special media
- **CFT, ELISA** etc. Usually together with serology of respiratory viruses
- Not possible to use antibiotics acting against the cell wall
- Effective **tetracyclins**; in children macrolides (i. e. erythromycin etc.)
- In *M. pneumoniae* vaccination attempts – is stage of experiment

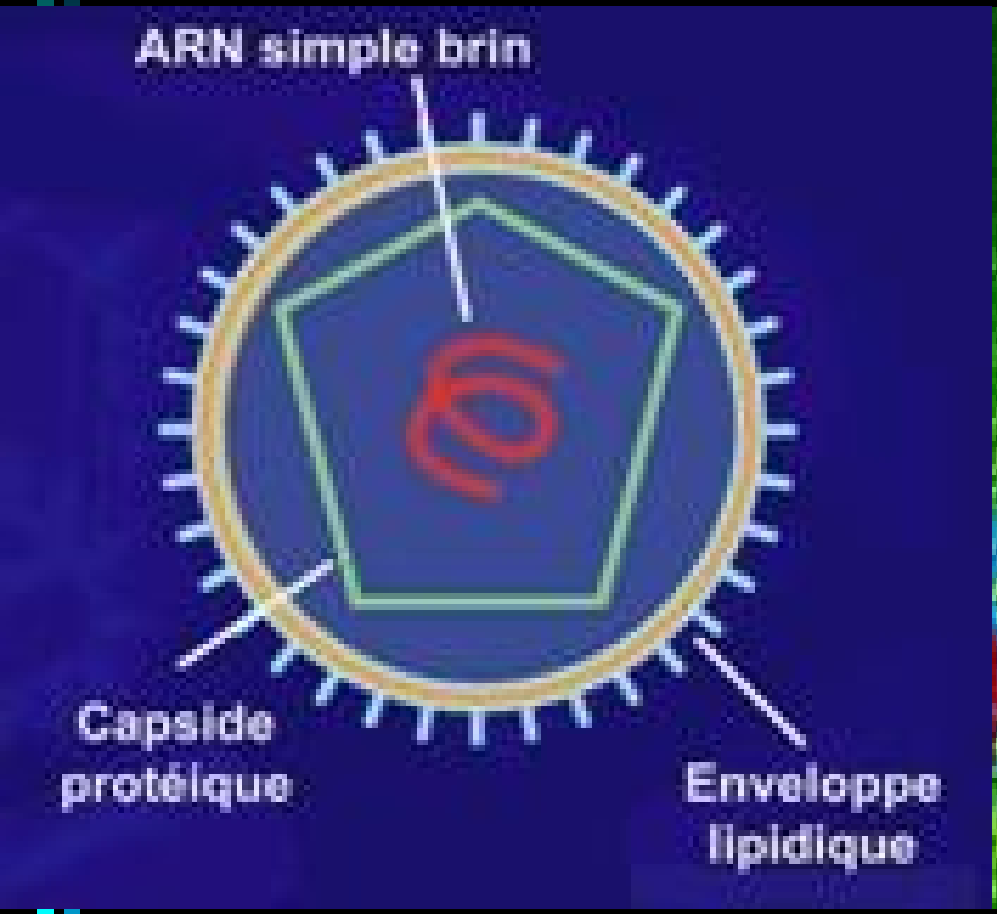


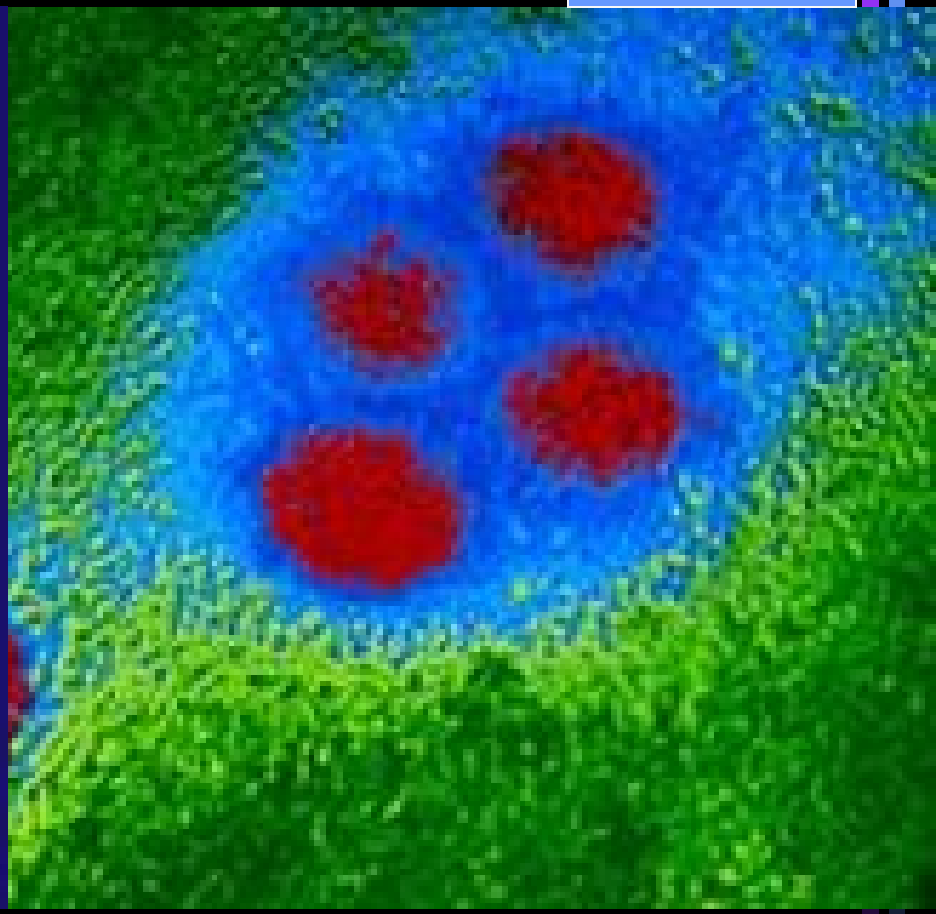
Virus of tick-borne encephalitis

Central European Virus of Tick-borne Encephalitis

- **RNA virus, belongs to flaviviruses**
- **Tick borne encephalitis** often infects children, heavy symptomas are mostly in adults. Adults paradoxically rarely let themselves **vaccinated**. First phase is „flu-like“, second has meningeal or cerebral symptomas. Letality is 1–5 %.
- It is a typical **arbovirus** (**ar**thropod **bo**rne virus), source of infection are rodents
- **Diagnostics** is mostly indirect diagnostics – CFT, HIT, ELISA. Eventually direct diagnostics virus isolation on newborn mice, eventually PCR

Virus of Tick-borne Encephalitis





http://vietsciences.free.fr/khaocuu/nguyenlandung/virus01.htm

Some viral properties

important for diagnostics

Among viral properties

- We know already, that viruses are acelullar particles, containing DNA or RNA in a nukleocapsid, and eventually also containing viral envelope
- A RBC-agglutinating agent may take part in this envelope. This effect was already seen in J09 practicle in **haemaglutination** inhibition test. Simillar is also ability of **haemadsorption**
- Other properties: **Virus needs alien cells.** Such cells are found e. g. in a tissue culture or in structures of fertilized egg with a chicken embryo

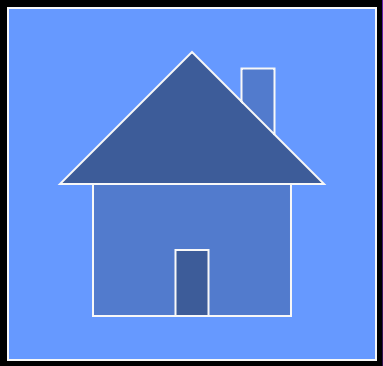
Survey of virological diagnostics

Virological diagnostics

- Microscopy: electronoptical, optical only to examination of somenting, that viruses do in vivo / in vitro (inclusions, cytopatic effect)
- Culture → isolation Requires living cells.
- Biochemical identification is not possible
- Animal experiment here equal to izolation
- Detection of DNA – in viruses > bacteria
- Detection of Ag in specimen – very common
- Indirect diagnostics – usually basis of the entire diagnostics

Influenza – diagnostics

- Diagnostics has **epidemiologic importance** (a proof, that the agent is the true influenza)
- **Direct influenza diagnostics** (sampling: nasopharynx washing with a special medium)
  - detection of **viral antigen**
  - **isolation on amniotic fluid** (virus then detected by Hirst test)
  - **isolation on monkey kidney cells**
  - detection of viral RNA using **PCR**
- **Indirect influenza diagnostics**
  - classical examination – pair sera, CFT, HIT
  - ELISA – IgM, IgA



Microscopy in virology. Viral isolation

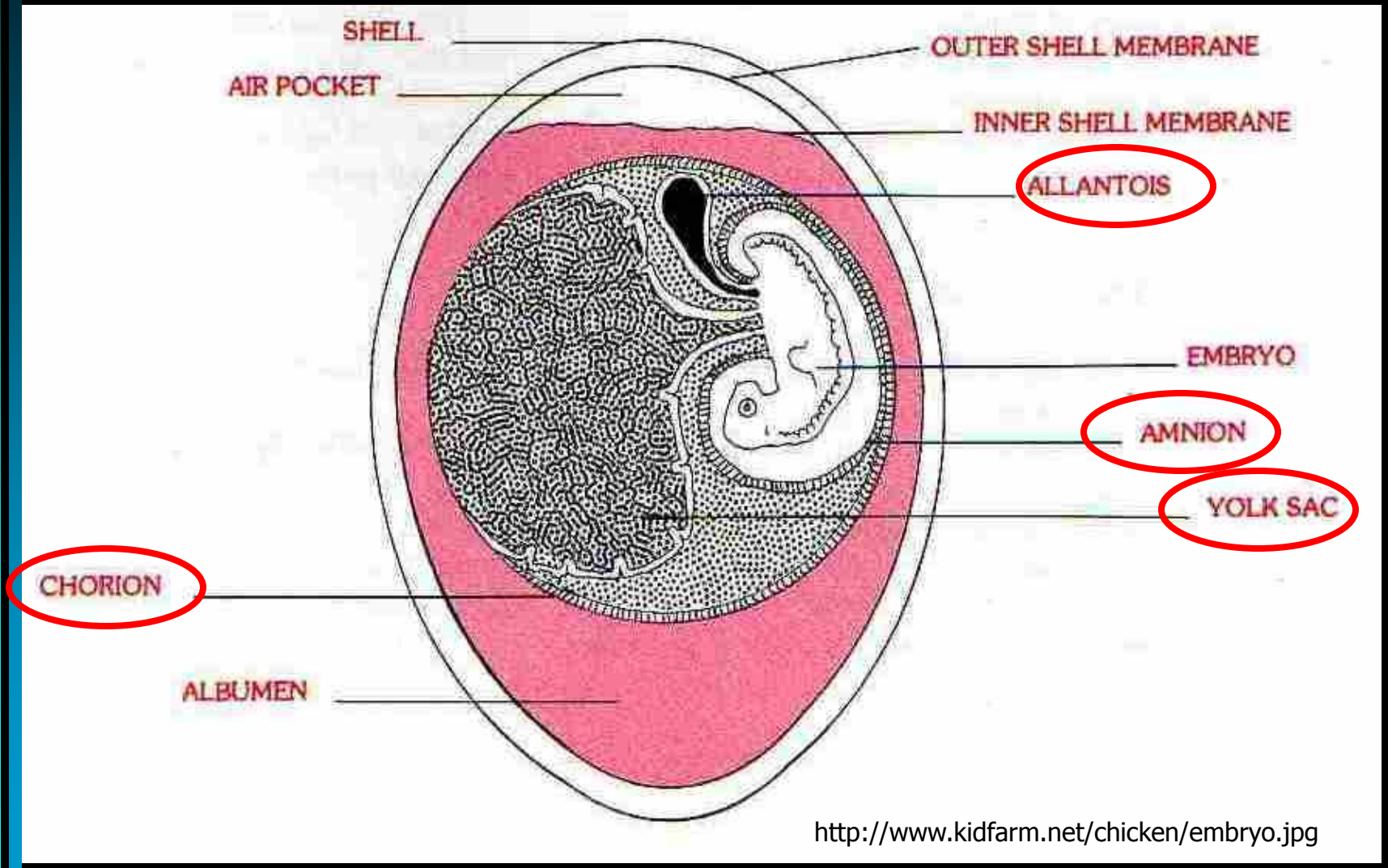
Microscopy in virology

- Elektronoptic microscopy is suitable for observation of majority of viruses, but it is very expensive and not available enough
- Optical microscopy may be used
  - To observe large viruses (poxviruses)
  - To observe cellullar inclusions in vivo (Negri bodies in rabies/lyssa)
  - To observe cytopathic effects in vitro (various viruses)

Viral isolation

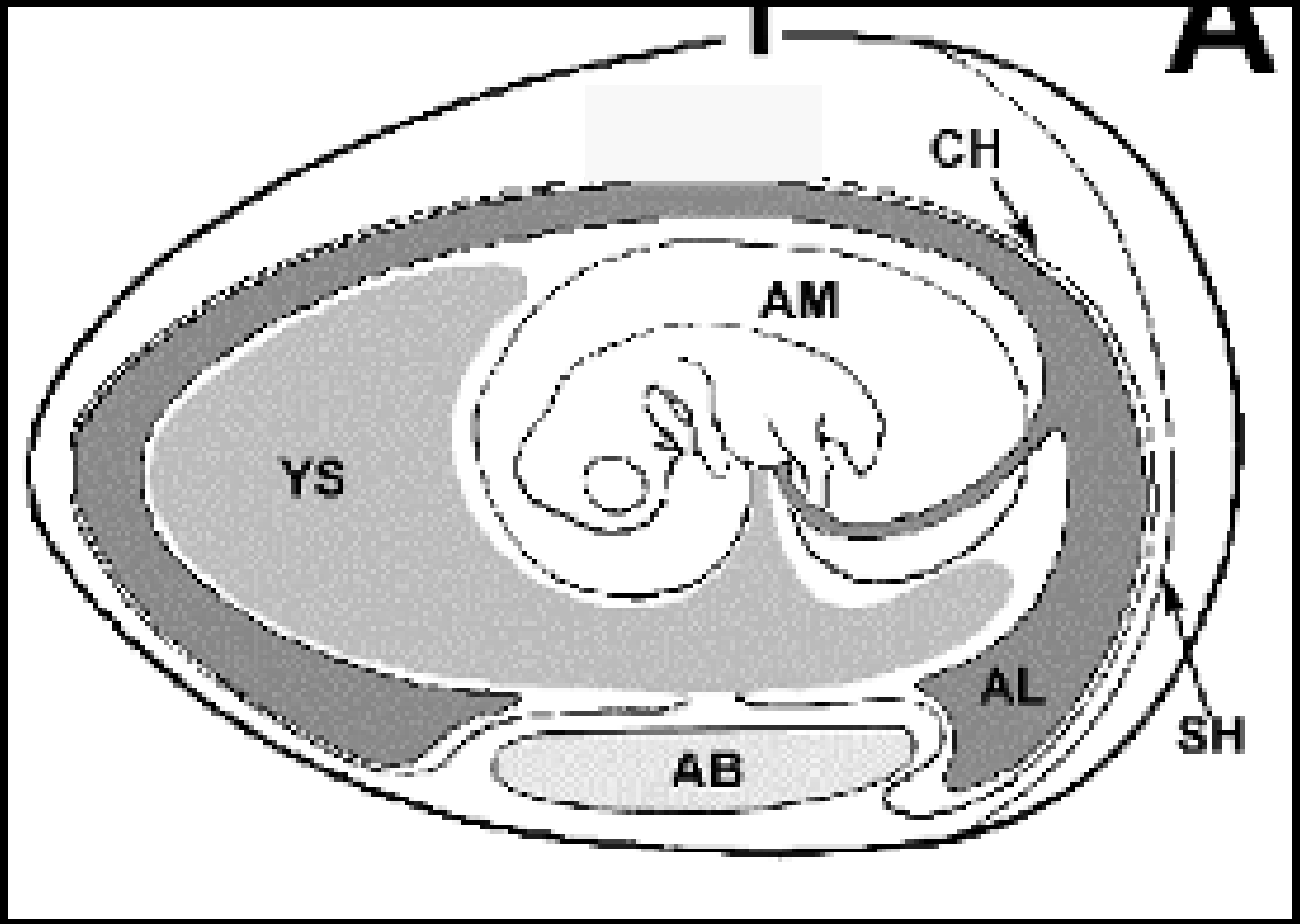
- Animal now less commonly. Typical animal is a suckling baby mouse.
- Fertilized egg is a classical method:
  - Amniotic sac
  - Alantoic sac
  - Yolk sac
  - Chorioallantoic membrane (only here sometimes a visible result – so called pocks)
- Tissue cultures: LEP, HeLa, monkey kindney and various other. Some viruses perform a cytopathic effect (CPE) on tissue cultures, but some viruses do not.

Fertilized egg and its parts



http://www.kidfarm.net/chicken/embryo.jpg

Another picture of the fertilized egg



SH – shell

AB – albumen

http://www.scielo.cl/fbpe/img/bres/v38n4/fig02.gif

AM – amniotic sac, YS – yolk sac, AL – allantois

CH – chorioallantoic membrane (CAM)

Isolation of viruses etc. on an egg

- **Amnitic sac**, surrounding the embryo, is used often, e. g. in influenza diagnostics
- **Allantois**, used by embyo as cavity for waste, is especially in older embryos easilly accessible. It is not very rich in nutrients
- **Yolk sac** is used for chlamydial diagnostics (they are bacteria, but simillar to viruses in properties)
- **Chorioallantoic membrane** serves to culture mostly poxviruses and herpesviruses
- *At production of a vaccine, the virus is cultured on allantois (afer several passages in amniotic fluid)*

Practical viral agglutination on fertilized egg
Ovoscope

- In time of technically fantastic machines, understandable for less and less technicians, a nice classical aparatus is **ovoscope**. It consists of a wooden bottle, a bulb and a shiftable wood with two holes.
- One is round to place an egg standing
- The second is oval to place an egg laying

Virus application to amniotic sac: how to do it

- Use ovoscope to see the air membrane
- Cut the shell next to air membrane
- Apply alcohol to paper membrane
- Use your ovoscop again
- Using needle, try to „stick the eye of the embryo" (it runs away anyway)
- Apply the virus inside
- Recap the egg and incubate for several weeks

How can I be sure that a virus is present?

- Bacteria at culture form visible colonies, or at least they make the broth turbid. On the other hand, in viral isolation the result is visible only sometimes (CPE, pocks), commonly the result is not visible
- Detection of an isolated viruses should be performed
- In viruses from **amniotic fluid**, mostly Hirst test is performed – detection of **viral ability to agglutinate erythrocytes**
- In viruses from **cell cultures** (e. g. virus of influenza from monkey kindey cells), we use rather **test of viral haemadsorption**

Hirst test practically

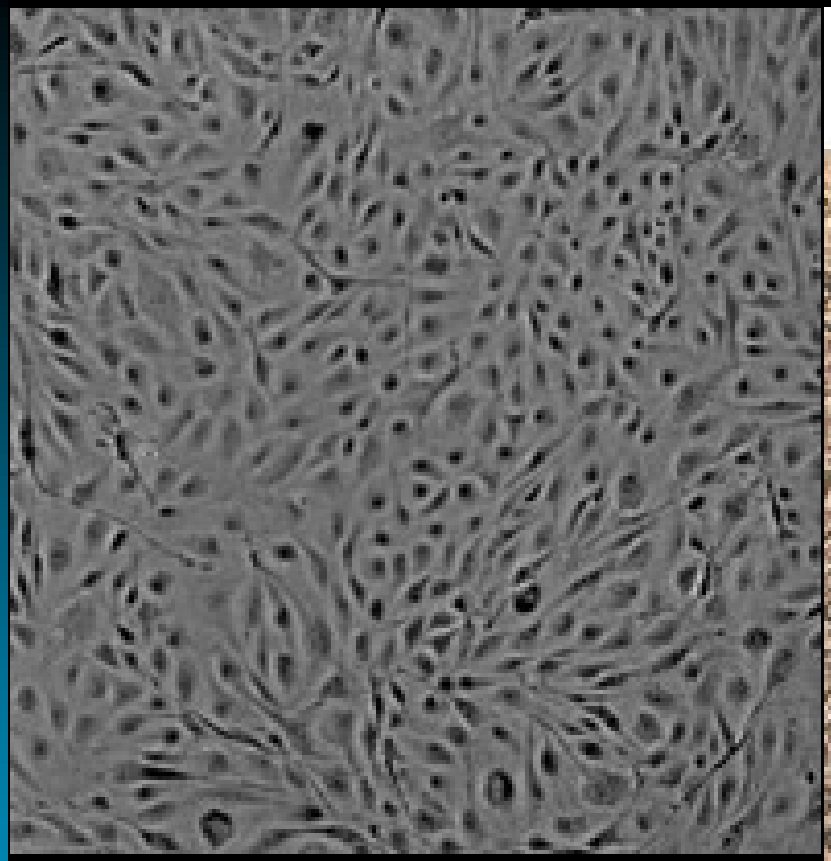
S = saline
V = virus (amniotic fluid)
E = erythrocytes

0.2 ml S	0.2 ml S	0.2 ml S	0.2 ml S	0.2 ml S	0.2 ml S	0.2 ml S	0.2 ml S
-----	0.2 ml V	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
	mix, pipette	mix, pipette	mix, pipette	mix, pipette	mix, pipette	mix, pipette	mix, pipette
0.2 ml E	0.2 ml E	0.2 ml E	0.2 ml E	0.2 ml E	0.2 ml E	0.2 ml E	0.2 ml E

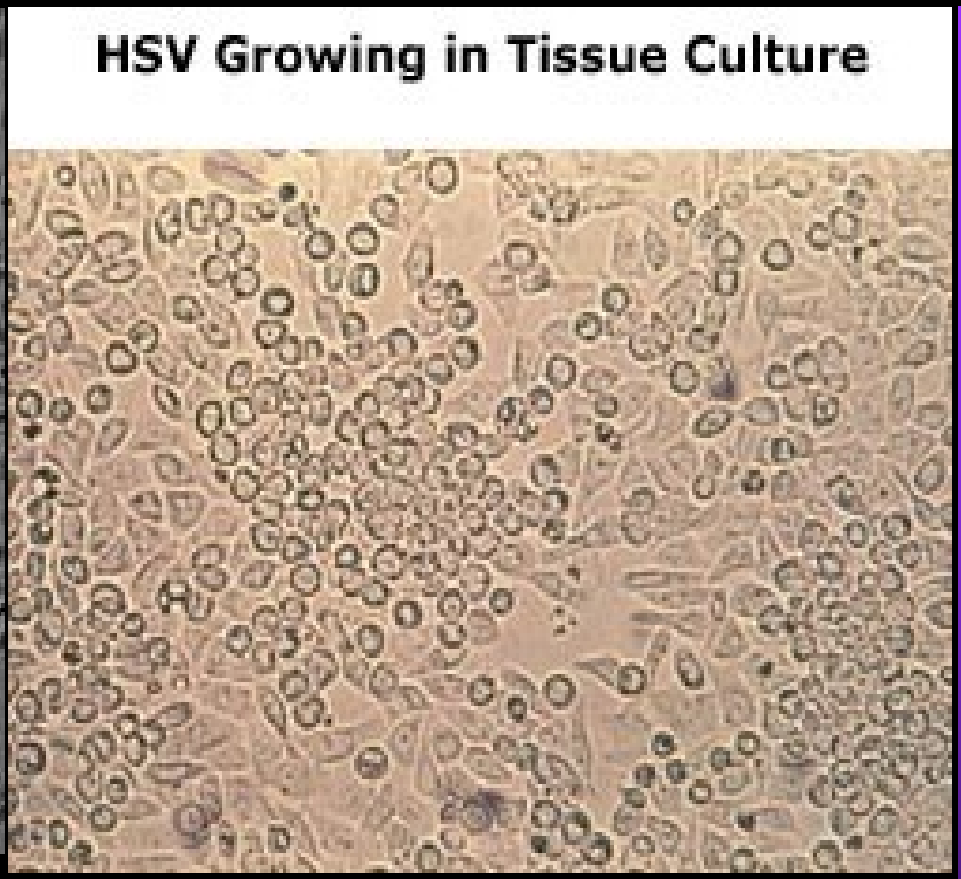
to disinfection

Cytopathic effect (CPE) on cell cultures

- Only rarely a cytopathic effect of a virus to a cell makes a **change of metabolism**, that could be observed as a macroscopically visible colour change after addition of an indicator (as we have seen in the virus neutralisation test)
- More commonly, CPE can be seen **in a microscope**:
  - the cells become round
  - desmosomic inter-cell connections are lost
  - the cells lose their one-direction orientation
  - globally, chaos replaces the order
- **Many viruses do not produce any cytopathic effect at all**



http://cmir.mgh.harvard.edu/cellbio/cellculture.php?menuID_=122



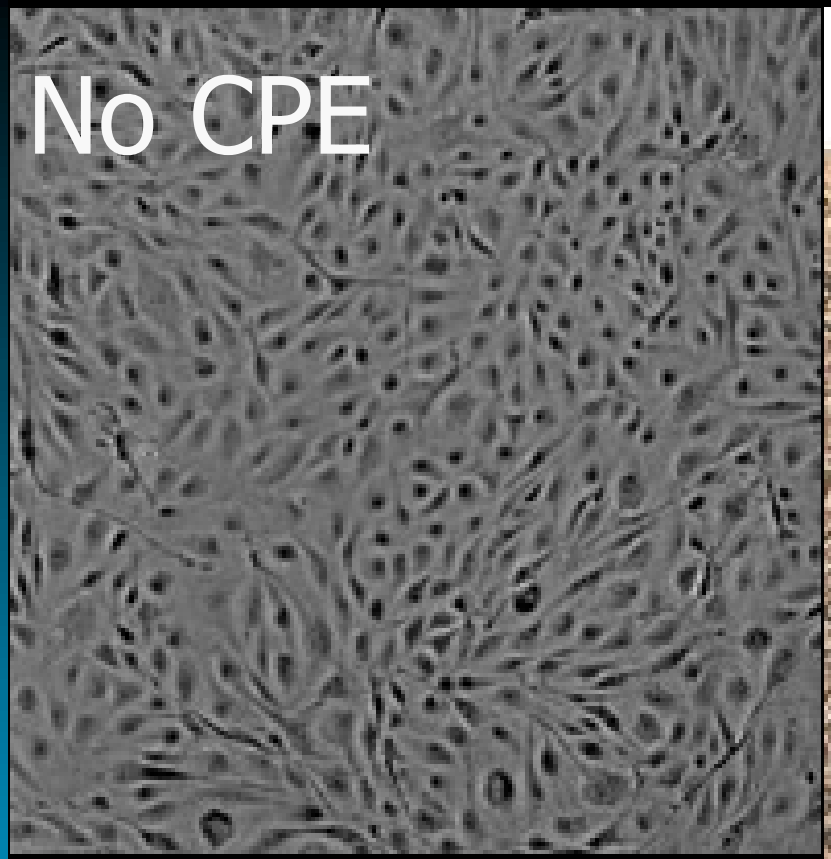
www.herpesdiagnosis.com/diagnose.html

You will see the same picture once again later

(HSV is Herpes Simplex virus – HSV 1 causing mostly herpes labialis, HSV 2 herpes genitalis)

Practical observation of cell cultures

- Put the entire test tubes to a microscope, trying to focus the inner surface.
- Maybe you will see cell cultures, some of them maybe even with a cytopathic effect
- An experienced eye recognizes various types of cell cultures, but also various types of eventual cytopathic effects



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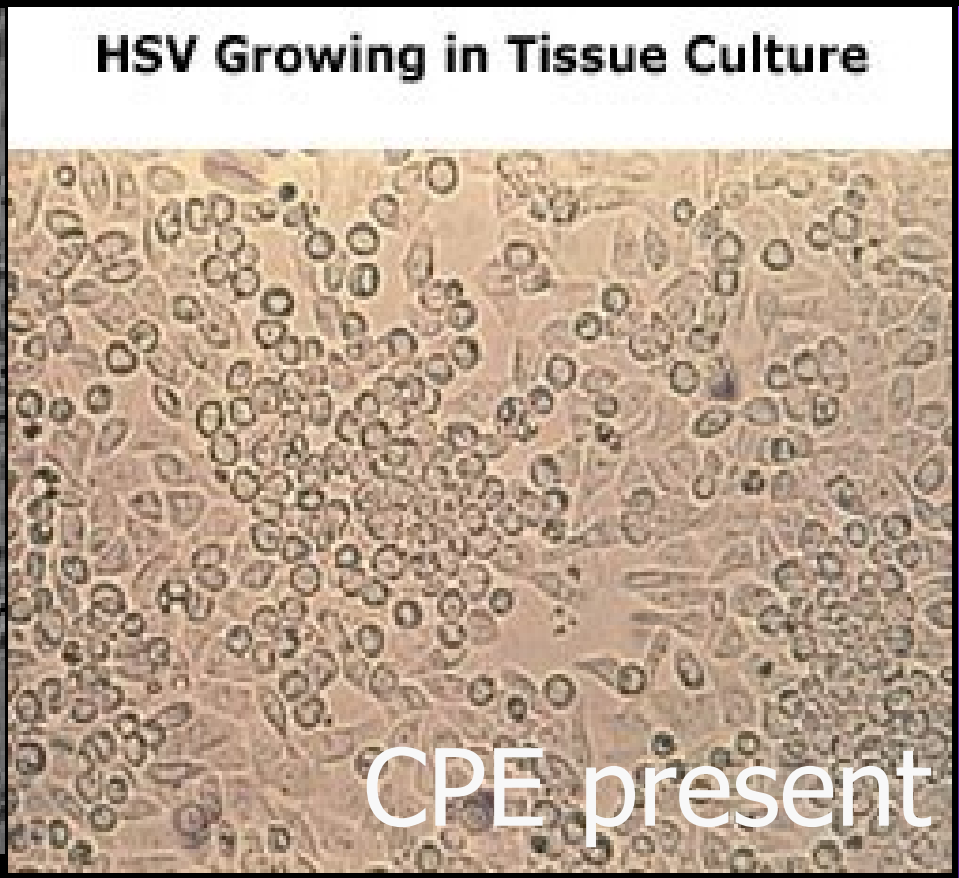

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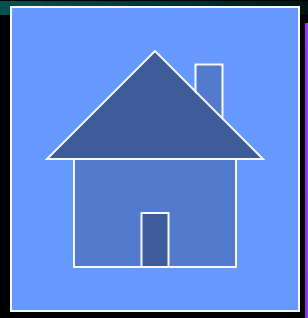
Shell-vials techniques



- Those are techniques of **quick culture**
- Inoculum is centrifuged inside of a **tissue culture growing on a round coverslip**
- Multiplied virus is detected using **immunofluorescence with monoclonal antibodies**
- During **24 h after material admission** everything is done (while classical culture durates several weeks)

Indirect diagnostics of viruses

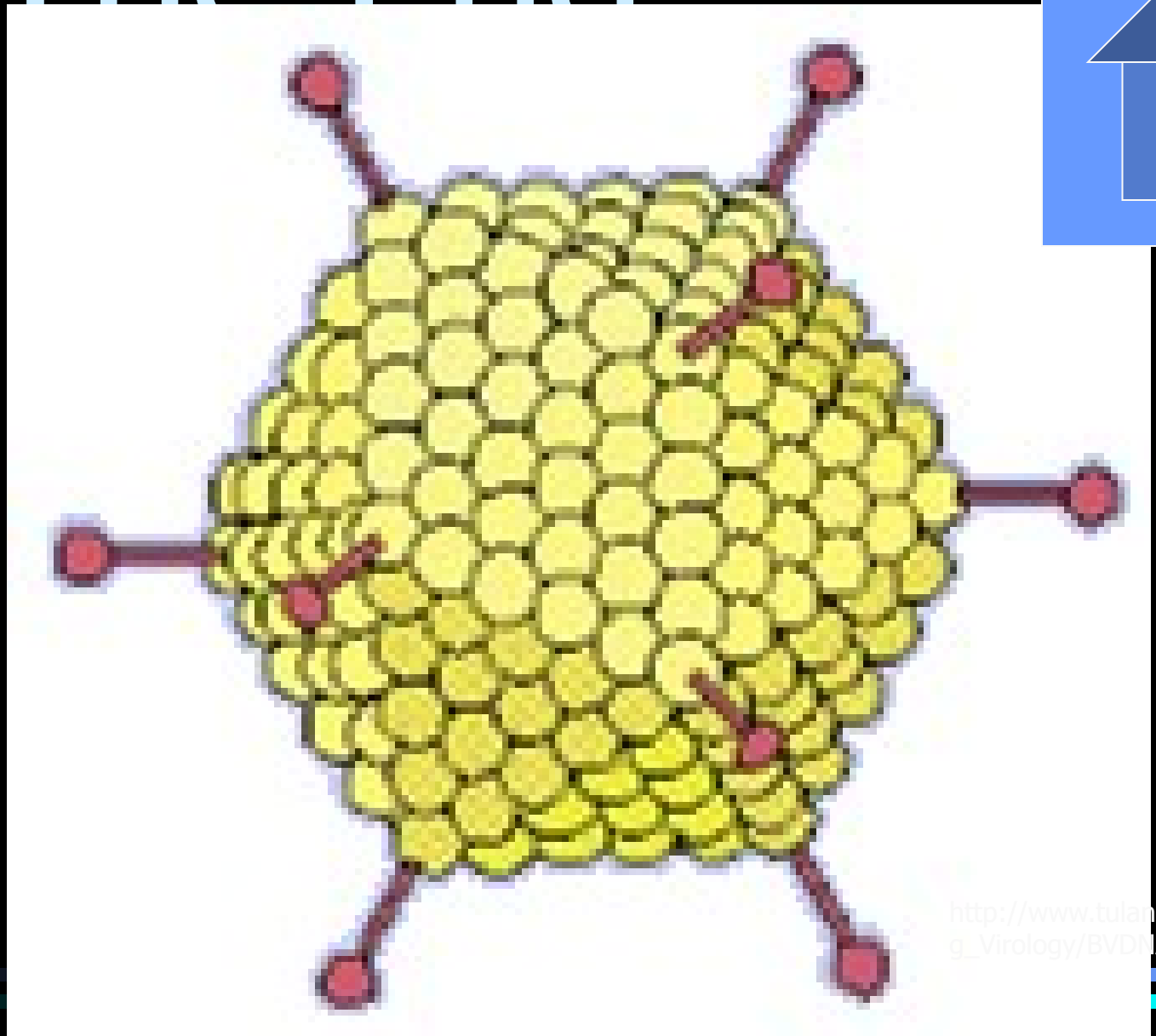
CFT in respiratory viruses – example

- The aim is to found, which of six tested agents is responsible for mometnary respiratory problems of our patient
- Positive: absence of haemolysis → RBC sedimentation to the bottom of the well
- Negative: haemolysis („strawbery lemonade")
- Titer = highest serum dilution with still positive reaction
- Fourfold increase/decrease of titer is supposed to be significant for running infection when using pair sera

HIT – tick borne encefalitis (example)

- Besides CFT, HIT is another classical method to detection of this virus.
- Positive: blocation of viral agglutination (= sedimentation of RBCs to the bottom).
- Negative: RBC agglutination („potato like" fotmation in the well)
- Titer = highest serum dilution with still positive reaction
- Fourfold increase/decrease of titer is supposed to be significant for running infection when using pair sera

The End



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