General virology and diagnostic methods in virology

FNM-H

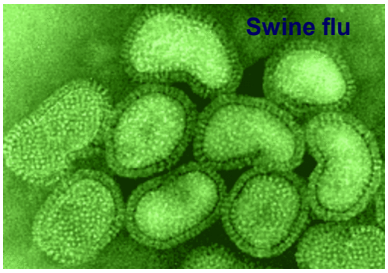
What is virus?

It is a submicroscopical pathogen containing the nucleic acid and proteins, which infects and reproducts in host cells.

Proliferation and multiplication of the virus is possible only in infected cells.
Viruses do not have translation system (ribosomes and transfer RNA) necessary for proteosyntesis. That is the reason why proliferation is possible in host cells only (bacterias, animals and plants).

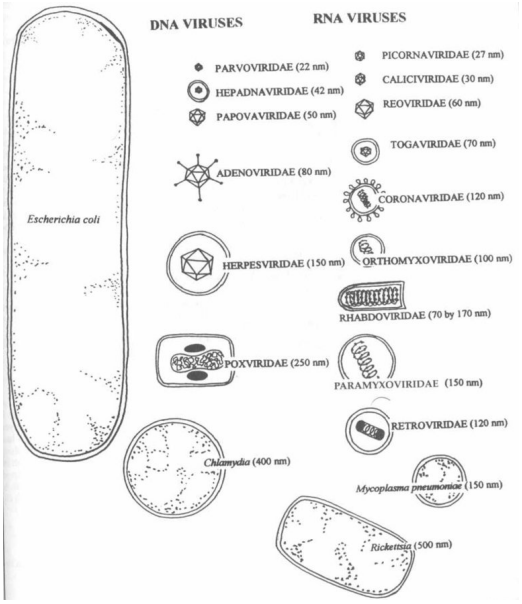
Some viruses (poxviruses, herpesviruses or rhabdoviruses..) contains enzymes important for viral reproduction inside the virions.

Virion is complete fully matured viral particule able to infect the cell.



Life is fight.

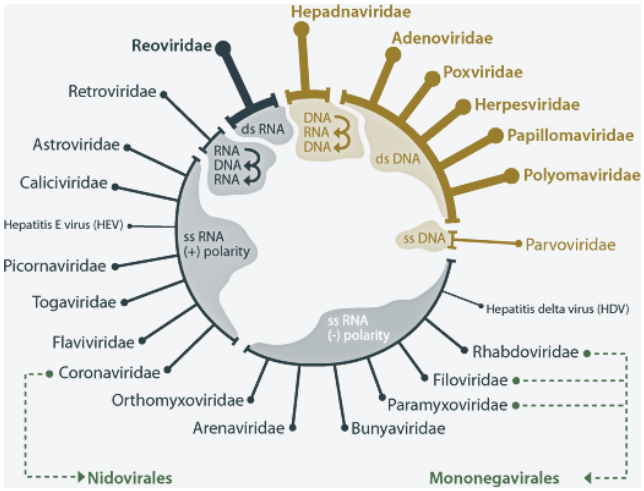
How they looks like?



http://www.epubbud.com/read.php?g=2RBLFKRP&tocp=5

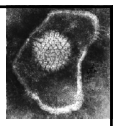
- Different capsid symetry
  (22 nm-250 nm)
- May be enveloped or non-enveloped
- For infection are important molecules on the viral surface which determines the cell receptors for virus binding and so specificity of viral infection for different cell types.

How they looks like?



- Coding nucleic acid can be both ss or ds and RNA and DNA
- + or - RNA
- Size of the genome is approx. between 3 kB and ≈ 200-300 kB
- Genome may be segmented or non-segmented
- Linear or circular

DNA viruses



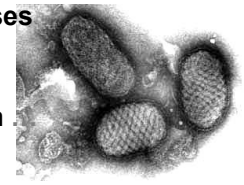
ds DNA

Herpesviridae
- α HSV 1 and 2, VZV
- β CMV, HHV-6 a 7
- γ EBV, HHV8

Adenoviridae → Adenoviruses (group A-F)

Polyomaviridae → BKV, JCV, WUV, KIV, SV40...

Papillomaviridae → Papillomaviruses



Poxviridae → Variolla virus, vaccinia, molluscum contagiosum

Hepadna → HBV

ss DNA

Parvoviridae
- → Parvovirus B19
- → Dependovirus

Anelloviridae → TTV, TTMDV, TTMV

DNA viruses

ds DNA

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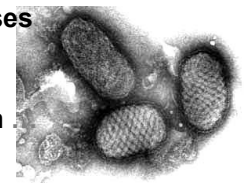

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Hepadna → HBV

ss DNA

Parvoviridae
- → Parvovirus B19
- → Dependovirus

Anelloviridae → TTV, TTMDV, TTMV

RNA viruses

ss RNA

- ***Ortomyxoviridae*** → **Influenza A**, **Influenza B**
- ***Paramyxoviridae*** → **Paramyxovirus** → **PIV 1-4**; **Morbillivirus**; **Pneumovirus** → **RSV, hMPV**
- ***Coronaviridae*** → 229E, NL63, OC43, HKU-1, SARS-CoV, MERS-CoV, SARS-CoV-2
- ***Picornaviridae*** → **Enteroviruses**; **Rhinovirus** → **HRV, EV, PV**
- ***Caliciviridae*** → Human caliciviruses → Norovirus, Sapovirus
- ***Astroviridae*** → Astrovirus
- ***Rhadboviridae*** → Lyssa virus

ds RNA

- ***Reoviridae*** → **Rotavirus**; **Orbivirus**

Does it make sense to know it?

Many viruses around

Enveloped / non-enveloped – diffrent physical and chemical stability
RNA / DNA - difference in stability and contagiousness

DNA

ds DNA

- *Herpesviridae* → α HSV 1 and 2, VZV; β CMV, HHV-6 a 7; γ EBV, HHV8
- *Adenoviridae* → Adenoviruses (group A-F)
- *Polyomaviridae* → BKV, JCV, WUV, KIV, SV40...
- *Papillomaviridae* → Papillomaviruses
- *Poxviridae* → Variolla virus, vaccinia, molluscum contagiosum

ss DNA

- *Hepadna* → HBV
- *Parvoviridae* → Parvovirus B19, Dependovirus
- *Anelloviridae* → TTV, TTMDV, TTMV

RNA

ss RNA

- *Ortomyxoviridae* → Influenza A, Influenza B
- *Paramyxoviridae* → Paramyxovirus → PIV 1-4; Morbillivirus; Pneumovirus → RSV, hMPV
- *Coronaviridae* → 229E, NL63, OC43, HKU-1, SARS-CoV, MERS-CoV, SARS-CoV-2
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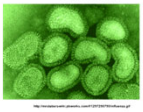


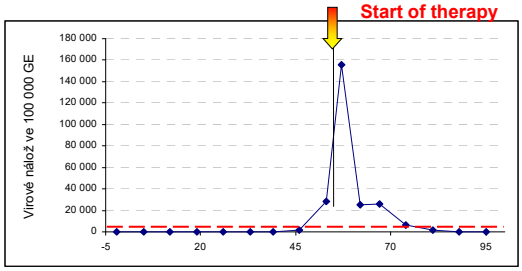
ds RNA

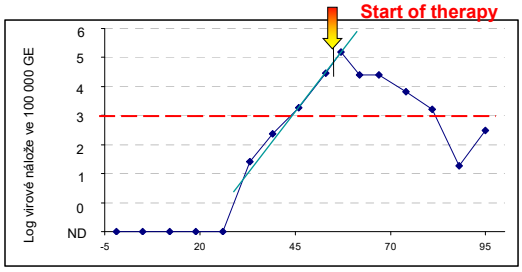
- *Reoviridae* → Rotavirus, Orbivirus

Bigger, ussually more genes.
Often – chronic and latent infections.
Immune systém manipulation.
Changes in cell cycle regulations genes – anti-apoptotic.

Smaller, less genes.
Rapid and acute disease.
Through TRL, IFN- bigger systemic impact of the infection.
Higher mutation rate.

Exponential virus proliferation





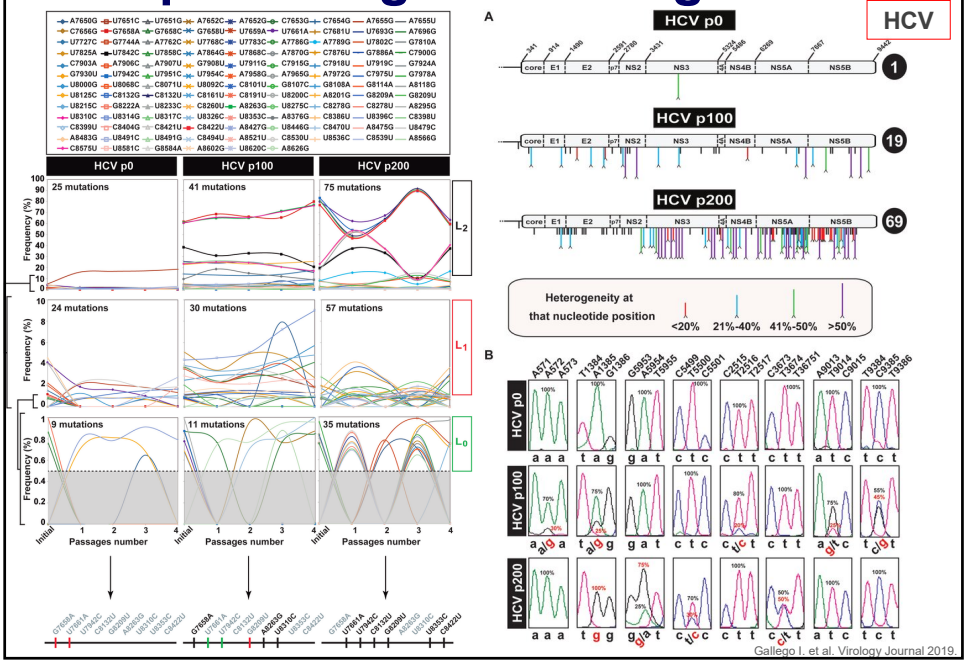
Patient with AML dg. after allo-HSCT

In vivo CMV doubling time

48-72 hours

Day	Diff. in days	CMV NVCs	Vypočítaná nálož do dalšího vyšetření při čase 48 hodin
19	7	0	
26	7	0	
33	7	260	
39	6	2 300	~~2 980~~
46	7	18 700	~~37 500~~
53	7	281 700	~~224 400~~

Rapid changes in viral genome



Gallego I. et al. Virology Journal 2019.

Rapid changes in CMV genome

Whole genome sequencing – NGS.

Human Cytomegalovirus Intrahost Evolution – A New Avenue for Understanding and Controlling Herpesvirus Infections

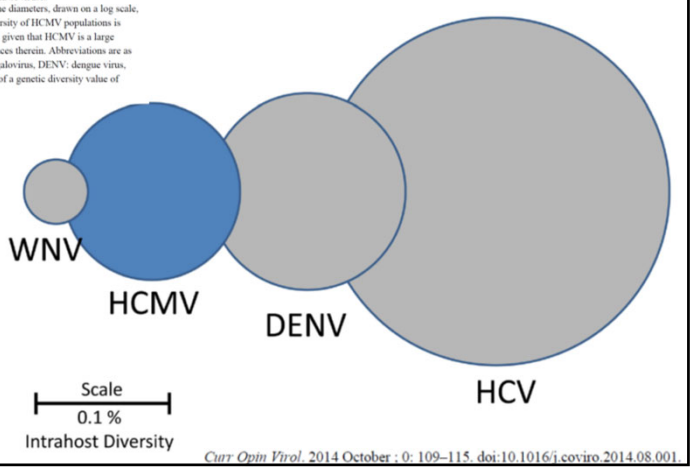
Nicholas Renzette[1], Laura Gibson[2], Jeffrey D. Jensen[3,4,5], and Timothy F. Kowalik[1,6,*]

Figure 1. HCMV Intrahost genetic diversity as compared to RNA viruses

Viral intrahost diversities are represented as circles with the diameters, drawn on a log scale, representing reported values of diversity. The genetic diversity of HCMV populations is comparable to those of RNA viruses, an unexpected result given that HCMV is a large dsDNA virus. Values were obtained from [16] and references therein. Abbreviations are as follows: WNV: West Nile Virus, HCMV: human cytomegalovirus, DENV: dengue virus, HCV: hepatitis C virus. Scale bar represents the diameter of a genetic diversity value of 0.1%.

Re-infections in10%

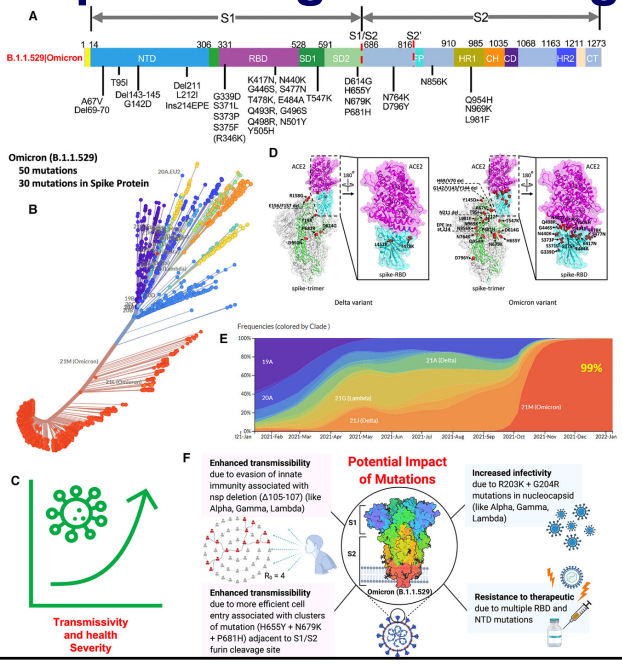
Predominant sequence in about 90% of viral population



Curr Opin Virol. 2014 October ; 0: 109–115. doi:10.1016/j.coviro.2014.08.001.

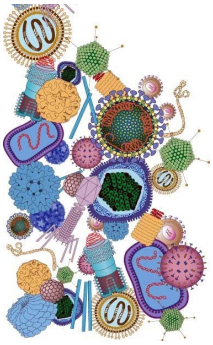
Rapid changes in viral genome

SARS-CoV-2



Mostafavi E. et al. MedComm.2022;3:1–8.

Balance in the patient



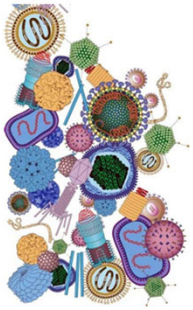
Immune system

(immunocompromised treatment, chemotherapy, …)

Pathogens

Lymfocyte regulated – viruses, mycoses

Balance in the immunocompromised patient



Immune system
(immunocompromised treatment, chemotherapy, ...)

Pathogens
Lymfocyte regulated – viruses, mycoses

In patient with steroids and immunosupressive therapy, symptoms do not have to develop completely, or might be modified including the rapid and severe course of infection. Vaccination may fail.

Always higher risk of symptomatic end-organ disease.

Detection methods in virology

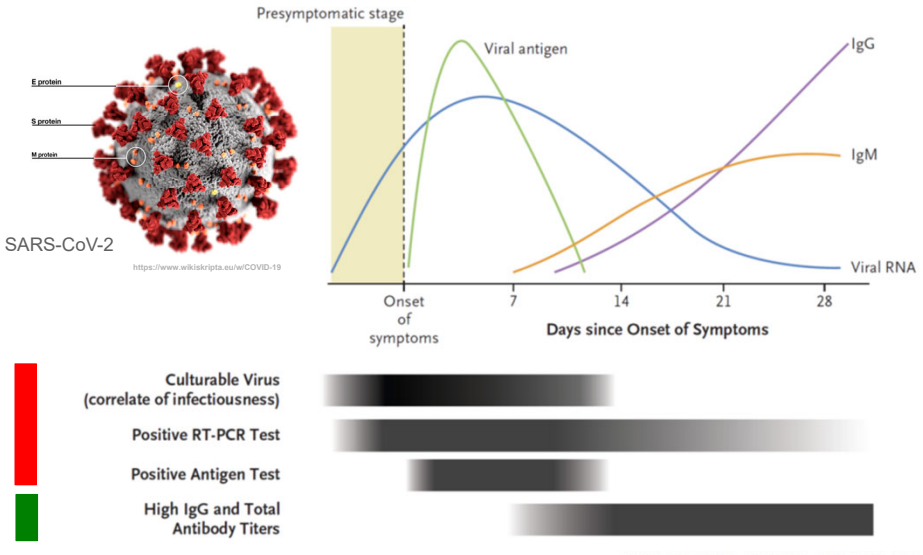
- Microscopic — **Direct detection**
- Cultivation
- Detection of the antigen
- Detection of the nucleic acid

- Detection of the antibodies
- (Signs of disease)

Indirect detection

Methods of the viral detection

Diagnostic window



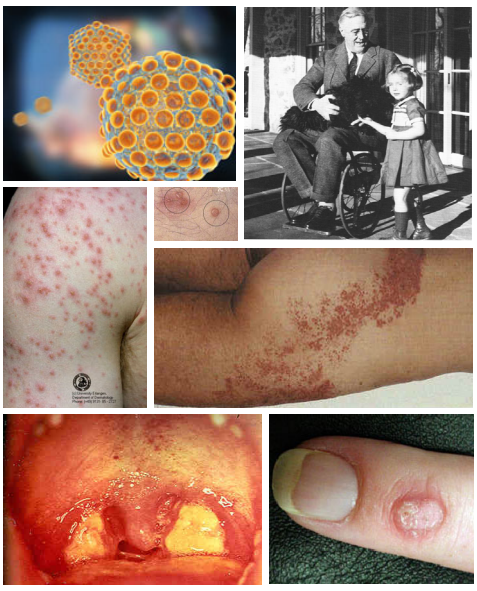
Methods of the viral detection - INDIRECT

Signs of the disease

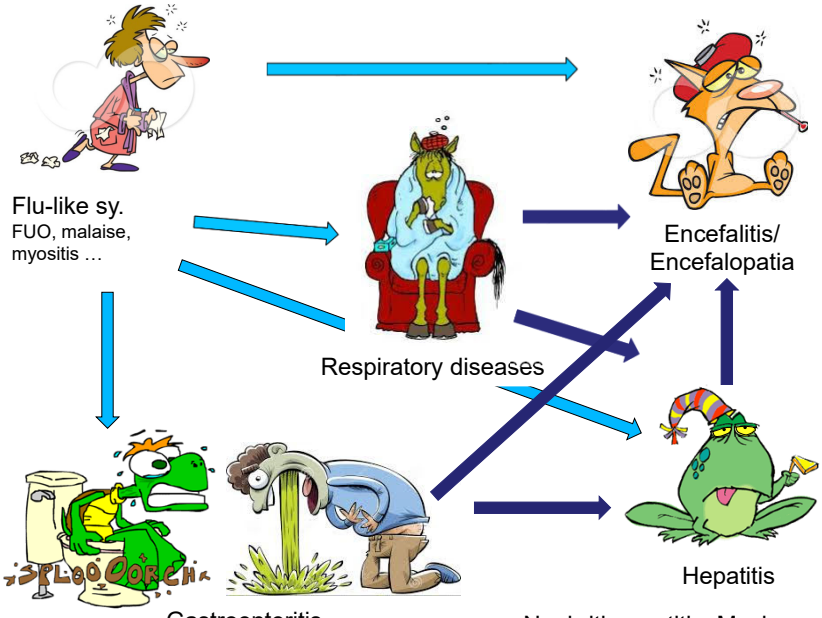
Clinical signs of disease leading to suspition of viral infection (poliomyelitis) were described first 3 700 BC in Egypt.

Typical signs are e.g. in:

- varicella
- zoster
- fully deleloped IM
- papillomaviral infection (wart)
- also in HHV-8 and other viral infections



Clinical consequences

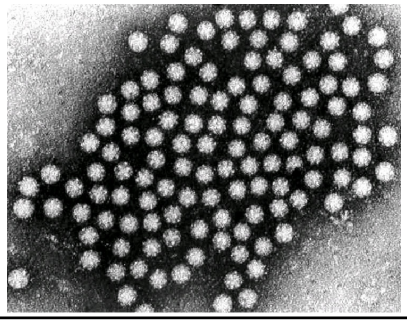


MAJOR ARTICLE

Astrovirus VA1/HMO-C: An Increasingly Recognized Neurotropic Pathogen in Immunocompromised Patients

Julianne R. Brown,[1,2] Sofia Morfopoulou,[3] Jonathan Hubb,[4] Warren A. Emmett,[3] Winnie Ip,[5] Divya Shah,[2] Tony Brooks,[6] Simon M. L. Paine,[7,9] Glenn Anderson,[7] Alex Virasami,[2] C. Y. William Tong,[4] Duncan A. Clark,[4] Vincent Plagnol,[3] Thomas S. Jacques,[7,9] Waseem Qasim,[5] Mike Hubank,[6] and Judith Breuer[1,8]

[1]Virology Department, Great Ormond Street Hospital for Children NHS Foundation Trust, [2]NIHR Biomedical Research Centre, Great Ormond Street Hospital for Children NHS Foundation Trust and University College London, [3]UCL Genetics Institute, University College London, [4]Virology Department, Barts Health NHS Trust, [5]Molecular and Cellular Immunology, [6]Molecular Haematology and Cancer Biology Unit, Institute of Child Health, University College London, [7]Department of Histopathology, Great Ormond Street Hospital for Children NHS Foundation Trust, [8]Department of Infection and Immunity, and [9]Birth Defects Research Centre, Institute of Child Health, University College London, United Kingdom



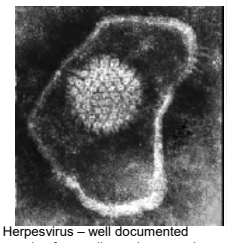
http://www.oardc.ohio-state.edu/saiflab/pictures/astro%20virus%204x4.jpg

Microscopic techniques

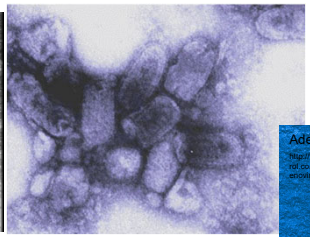
- Electronmicroscopic detection of virus
  - In liquid materials after virus concentration
  - In the tissues
  - Immunoelectron microscopic detection after signing of virus with specific antibody
- Immunohistochemical detection of virus in the cells
  - Methods for histology testing of biopsies
  - Cytological technique

Electronmicroscopic evidence

First photography of virus was published in 1939. Further development of electronmicroscopy established this technique in rutine diagnostic of viral infections. Different morphology and size of viral particules makes this technique still very useful in clinical detection.

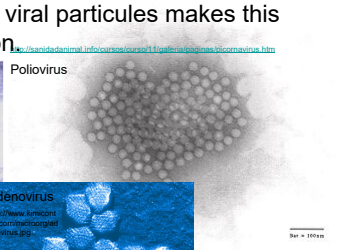


Herpesvirus – well documented envelop from cell membrane and capsid.

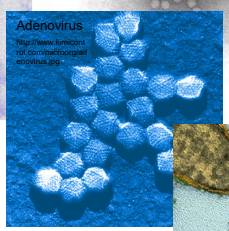


Rhabdovirus – rabies virus

http://www.stanford.edu/group/virus/rhabdo/2005/Rabies%20Virus.htm

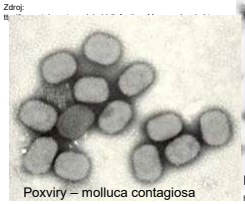


Poliovirus

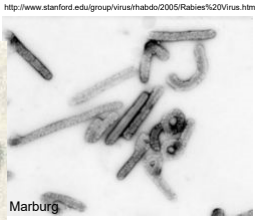


Adenovirus

http://www.lumicontrol.com/mellcoorgtadenovirus.jpg -

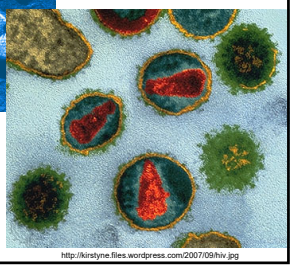


Poxviry – molluca contagiosa



Marburg

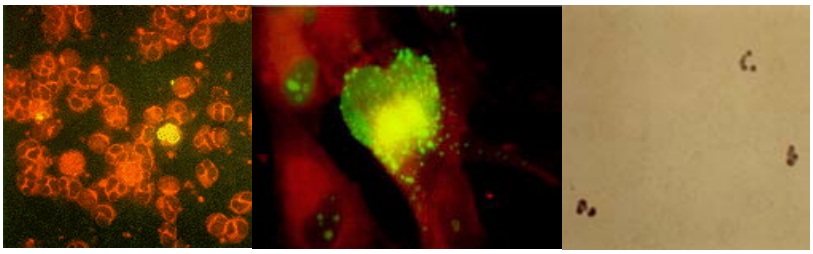
http://content.answers.com/main/content/wp/en-commons/thumb/9/91/250px-Marburg_virus.jpg



http://kirstyne.files.wordpress.com/2007/09/hiv.jpg

Immunohistochemical detection of viral proteins

At the present time, detection of viral proteins is based on using of monoclonal antibodies fluorescent microscopy.

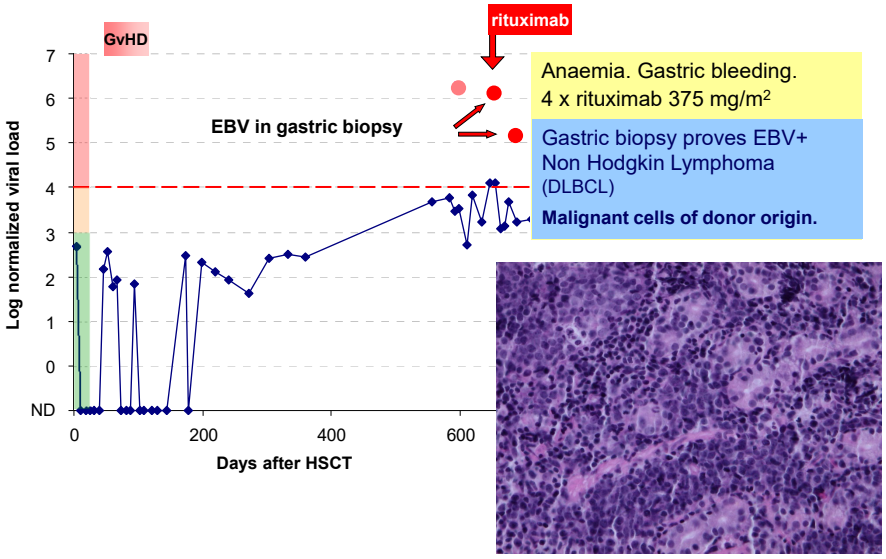


Detection of pp65 CMV antigen using fluorescent microscopy. Zdroj: ttp://home.teleport.com/~bobh/InfectiousMononucleosis.htmhttp://www.argene.com/pictures_gallery/zoom_images_ang/CMV_Antigenemia_perox.php

Using of microscopical techniques

- Histochemical detection
  - Especially during pathological testing
- Electron microscopy
  - For particular types of samples and viruses
  - Lower sensitivity comparing to the cultivation and PCR
- Optical microscopy
  - Might be useful supplemental technique
    - Signs of inflammation without bacterias suggests viral ethiology

Localised EBV-LPD (NHL)



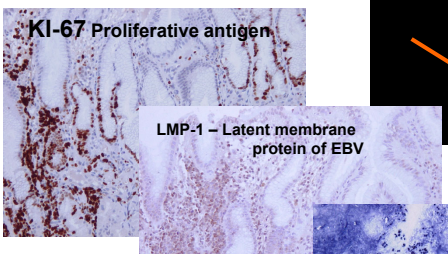
Anaemia. Gastric bleeding.
4 x rituximab 375 mg/m^2

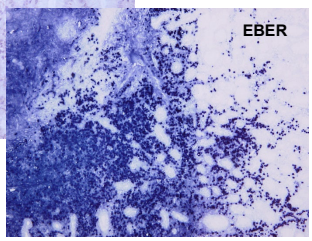
Gastric biopsy proves EBV+
Non Hodgkin Lymphoma
(DLBCL)

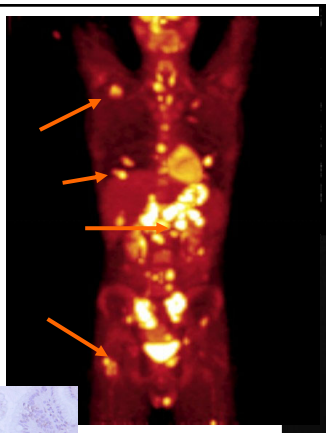
Malignant cells of donor origin.

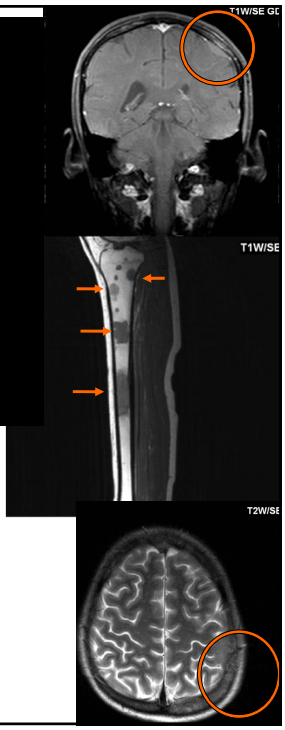
Treatment according to the **BFM NHL 2004** protocol.

During last block of chemotherapy Pseudomonas aeruginosa sepsis.









Methods of cultivation

- Cultivation on cell (tissue) cultures
  - Classic with cytopathic effect
  - Rapid with immunochemical visualiazation of the virus
- Cultivation on chicken embryos
- Test on the animal

Tissue cultivation

Pros

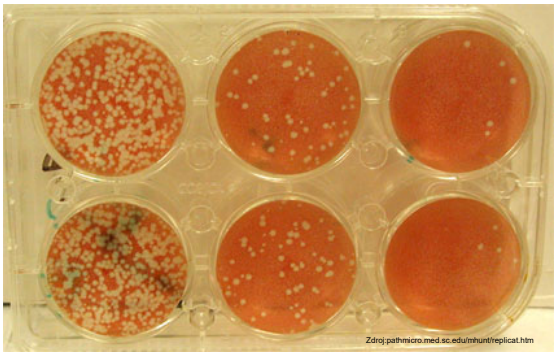
- Prooving a „living“ virus
- Ability to do additional tests
- Detection wider spectrum of viruses

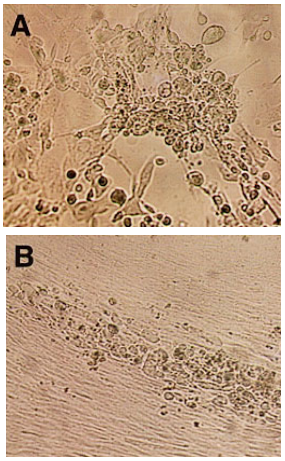
Cons

- Sensitive to transport conditions
- Some viruses are badly cultivated in vitro (longer time to detection)
- Difficulties in work with tissue monolayers (contamination with bacterias and mycoses)

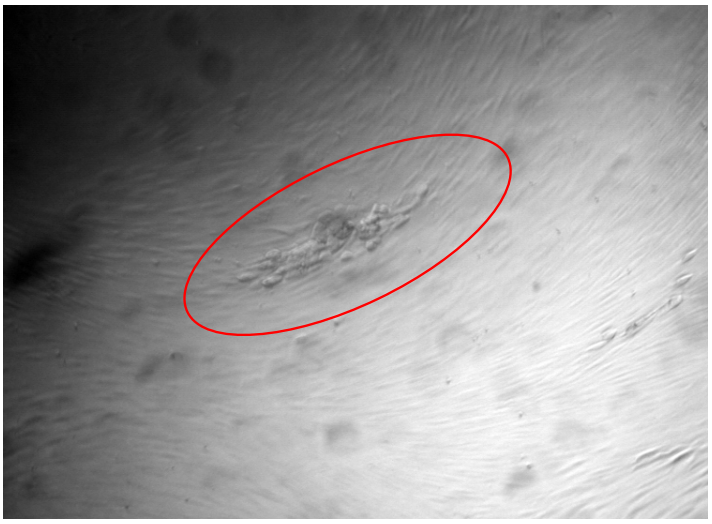
Viral cultivation

Additional possibility for viral detection is cultivation on tissue monolayers. J. Enders used it for the first time for poliovirus in 1949. Plaque forming assay was first used in 1952 by R. Dulbecco. Subsequently, plaque forming units (PFU) were established. Shell vial cultivation shortened the time.



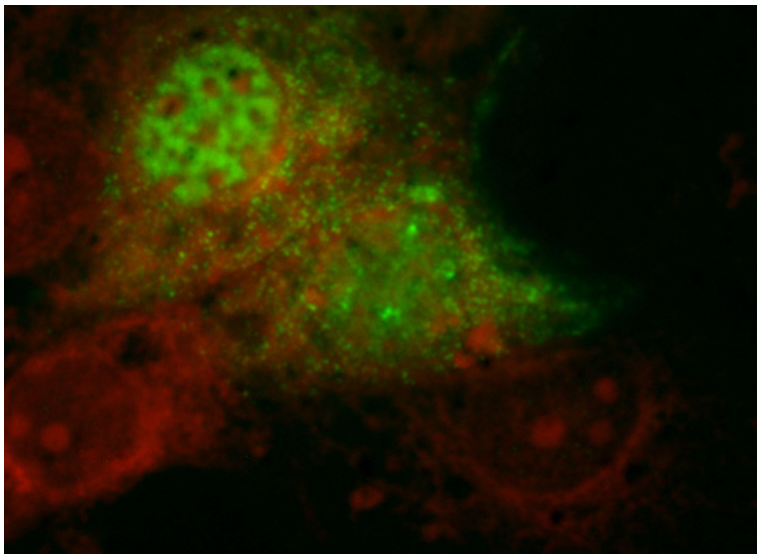


Cytopatic effect - CMV



Influenza A virus on tissue monolayers

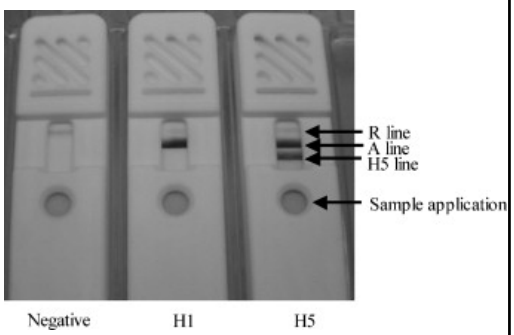
- monoclonal antibody stained with FITC



Methods of the viral detection - DIRECT

Viral antigen detection

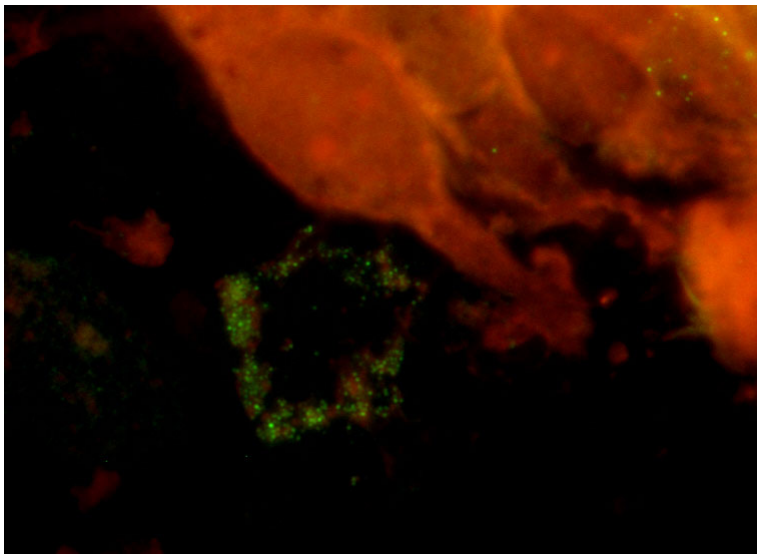
- Detection of single or only couple of pathogens
- Detection of presence/absence; sometimes possibility of (semi-) quantification.
- Based on antigen-antibody reaction



Sensitivity approx. about 30-40% compared to PCR (real ≈20%).

Price approx. 4-6 €

Antigen detection of Adenovirus in the lung tissue



Methods of the viral detection - DIRECT

Using of antigen detection

- In case of infection with defined clinical picture and only few possible pathogens (e.g. respiratory tract inf.).
- Infection necessary to be monitored in defined group of patients (e.g. CMV in the immunoxompromissed host).
- Infection, in which is antigen present regularly in huge amounts (hepatitis B).

Is antigen detection really easy?

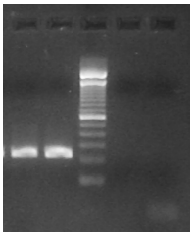
- Technically is antigen detection easy
- Difficult is interpretation of result
  - There is no „normal range“
  - Sensitivity of methods depend on type of used antigen – no standardisation
  - Immune system of every person react in a unique way.

Nucleic acid detection

- Detection of one or couple agens only
  - Amplification (PCR, NASBA) - sensitive
  - Without amplification (gene probes) – less sensitive
- Rapid and perspective

PCR reactions

- Qualitative
  - Basic diagnostics
  - Detection of presence/absence of single agens only
- Multiplex
  - Detection of more pathogens in a single reaction
  - Important is detection of product
- Quantitative
  - Competitive
  - Real - time PCR



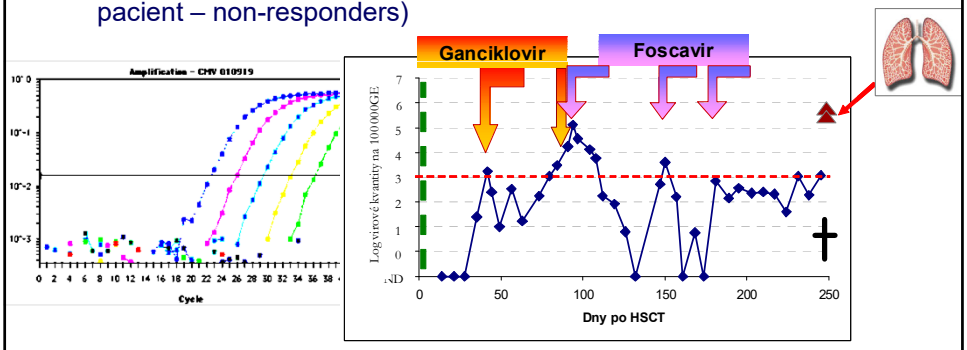
Using of PCR

- **Pros**
- High sensitivity
- rapid
- Highly specific
- Possibility of quantification

- **Cons**
- Sensitive for manupulation
- Detection of vial and non-vial agens
- Risk of inhibition and false positivity

Using of Real-time PCR

- Quantification of microbial agens to find diagnosis and prognosis
- Monitoring of patients in immunosupression (quick start of the treatment)
- Monitoring of virostatic treatment (detection of resistence in pacient – non-responders)

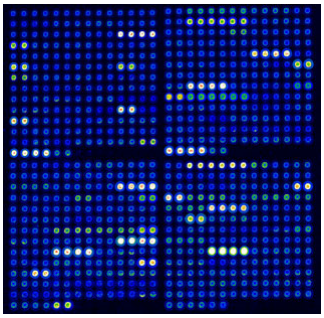


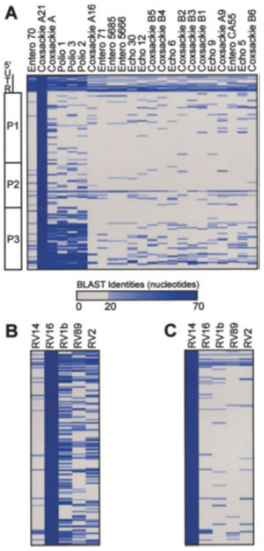
Sequencing and detection of agent according to the database

- Detection of nucleotid sequence according to the database
- Less useful in virology
- Quality of database matters
- At the present time more supplementary

Detection of nucleic acids using CHIP technique

Since 2000, there are first papers describing possibility of viral detection by CHIP technique.



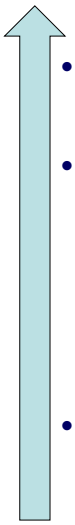


This approach was used also for discovery of two new human polyomaviruses WU and KI in 2007 which were isolated from respiratory tract.

Comparing of techniques – sensitivity

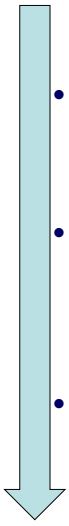
- PCR and cultivation amplified samples quantity of the agens - sensitive
  - PCR depends on type of techniques (primers, multiplex…)
- Cultivation is easily influenced by experience and type of agens (growth factors)
- Detection of antigens in sampled quantity only – less sensitive
- Microscopy is more or less for orientation only

Comparing of techniques – specificity



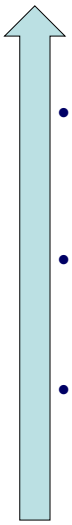
- Cultivation has minimum of false positive reactions
- PCR – depends on quality of detection and primers – detects all not only viable virus
- Antigen detection has lower specificity

Comparing of techniques – necessary time



- Antigen detection – results normally within 30 minutes
- PCR – result can be generated from dozens of minutes to couple of hours
- Cultivation – takes usually days to weeks

Comparing of techniques
– possibility of wide detection



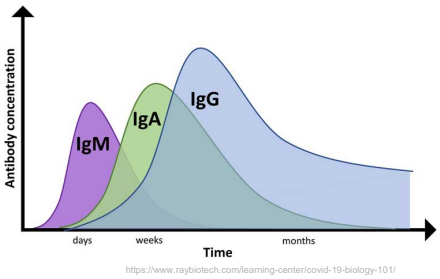
- Electron microscopy detect widest spectra of viruses (limited by staff experience)
- Cultivation can detect more viruses
- PCR and antigen detection detects only particullar pathogens (with exception of sequencing)

Methods of the viral detection - INDIRECT

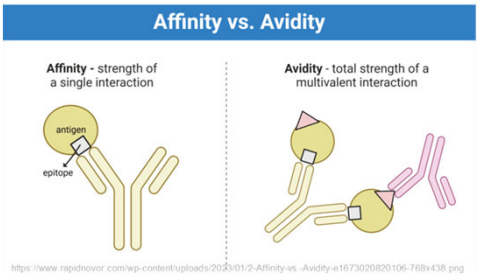
Detection of antibodies reflects only reaction of part of the immune system against infection.

Detection of antibodies

- Can detect immunoglobuline in different classes (IgG, IgM, IgA)
- At the beginning of the infection, there is no specific antibody production
- Not suitable for monitoring of the treatment



https://www.raybiotech.com/learning-center/covid-19-biology-101/



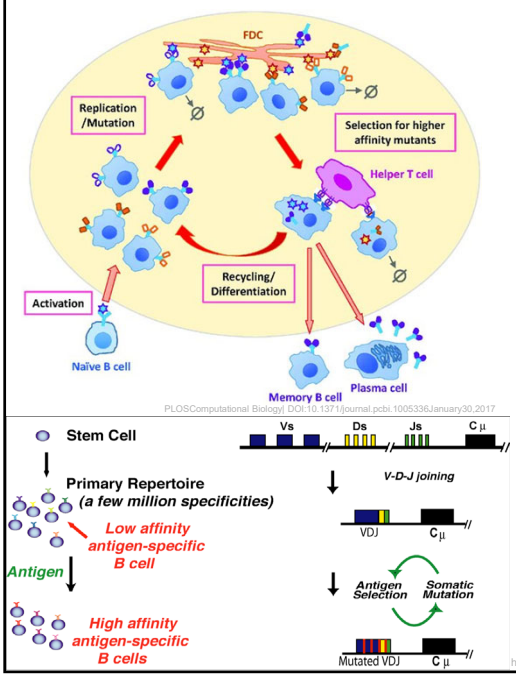
https://www.rapidnovor.com/wp-content/uploads/2023/01/2-Affinity-vs.-Avidity-e1673020820106-768x438.png

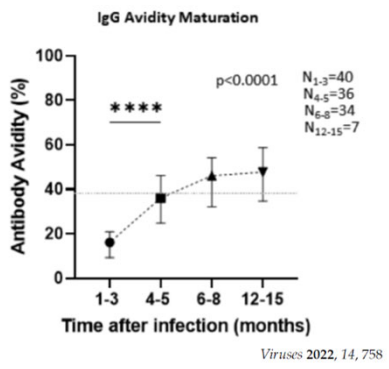
Main usage of the antibody detection

- Viral infections with huge systemic response (influenza, rubella, hepatitis)
- More severe bacterial infections (pertusse, syfilis)
- Systemic infections with single cell parasites (toxoplasmosis)

Limited or small impact of antibody detection

- Infections with intracellular bacterias (*Mycobacterium tuberculosis*)
- Local infections (uncomplicated salmonelosis, tonsilitis, urinary tract infections)
- Reactivation of persistent infections (herpes)
- Additional information for interpretation of positivity is **antibody avidity**





Classical methods of antibody detection

- Complement fixation
  - Good specificity, reasonable sensitivity, cheap.
  - Application: especially for respiratory tract infection
- Haemagglutinin inhibition
  - specific, reasonable sensitivity, cheap.
- Agglutination and precipitation
  - specific, less sensitive, cheap.

Major disadvantage is necessity of pair serum testing.

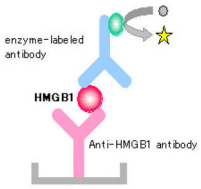
Immunochemical methods

EIA (enzyme immunoanalysis)

IF (immunofluorescence)

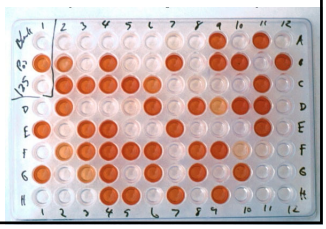
RIA (radio-immunoanalysis)

ELISA (enzyme-linked immunosorbent assay)



Advantage: very good reproducibility of resul, discrimination of immunoglobulin classes, high sensitivity

Disadvantage: more expensive, sometime unspecific results



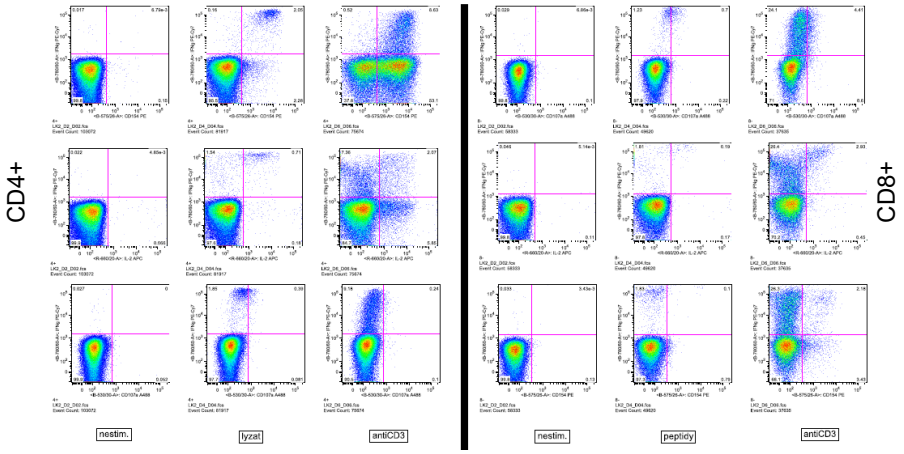
Why can antibody detection fail?

- Significant part of infection is destroyed by unspecific immunity
  (no activation of specific immunity)
- Detection has limited sensitivity, method is inappropriate or blood sample has been drawn too early
- Infection was caused by another than tested pathogen.

Detection of specific lymphocytes

Further step in detection of viral infection consequence using molecular biology. Detection of lymphocytes producing IFN-γ after antigen stimulation – by ELISPOT assay or flow cytometry.

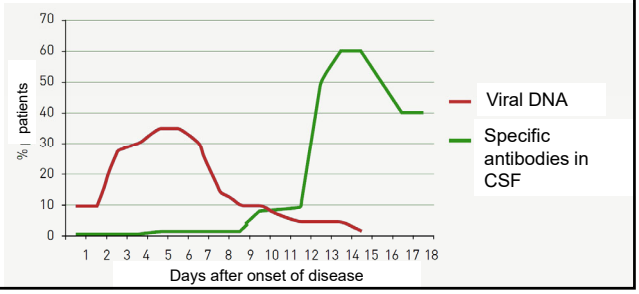
Detection of specific lymphocytes



Sampling of material for cultivation

- In an acute phase of infection
  - Smaller viability in latter phases
- Sampling from the place with highest pathogen concentration
  - Important is the knowlegde about pathogenesis of infection
- Vigorous sampling (to obtain enough material for testing)

PCR and antibody response against herpesvirus infection in CSF



Sampling for direct detection

- Antigen detection (and Nucleic Acid) without amplification)
  - Very important is sampling of vigorous volume of material in acute phase
- Nucleic acid detection with amplification
  - Can be used also in situation when number and viability of pathogens decrease (but there are also some limitations!)
  - There is necessary to use special clean sampling and transport sets (due to risk of DNA and especially RNA destruction)

Transport

- Cultivation
  - Transport medium according to the suggestion of the lab
  - Maintaining and transport at fridge temperature
  - Important is lenght of transport (up to 24 hours)
- Antigen detection
  - It is important to avoid sample destruction
  - Ideally immediate detection after sampling
- Nucleic acid detection
  - It is important to avoid sample destruction (destruction of NA or adding of inhibitors into the samples)

What is important for communication with the lab

- To know what I need
  (Ask a proper question – differential diagnostics)
- To know what and how quickly can be tested
- To know pathogenesis of infection and test the samples according to the phase of infection
- To be able to communicate with people from lab

What should good lab do

- Standart result in a reasonable time
- Express testing in severe clinical situations
- Consultation of diagnostical possibilities
- Interpretation of the result and advise with further steps in diagnostics and therapy
- Inform clinical staff about diagnostical impact of the result (sensitivity, specificity, negative and positive predictive value)

Before sampling a sample, you have to know what will be impact of the result for management in case of
positivity or negativity.
If there will be no difference in both cases – test is senseless.


Thank you for you attention
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