

General virology and diagnostic methods in virology



What is virus?

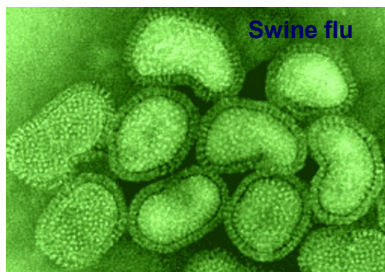
It is a submicroscopical pathogen containing the nucleic acid and proteins, which infects and reproduces in host cells.

Proliferation and multiplication of the virus is possible only in infected cells.

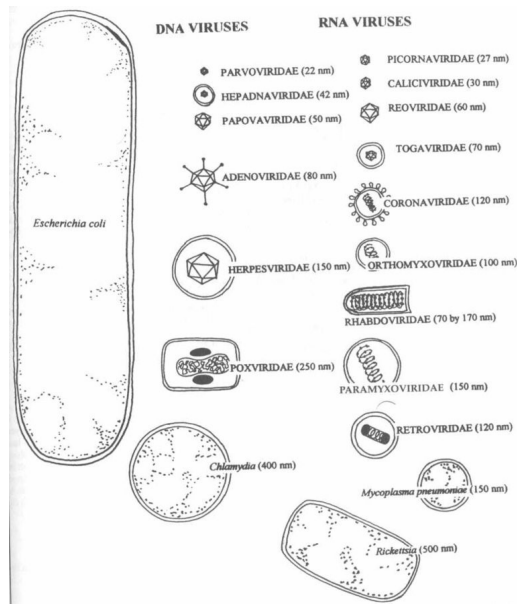
Viruses do not have translation system (ribosomes and transfer RNA) necessary for proteosynthesis. That is the reason why proliferation is possible in host cells only (bacterias, animals and plants).

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.



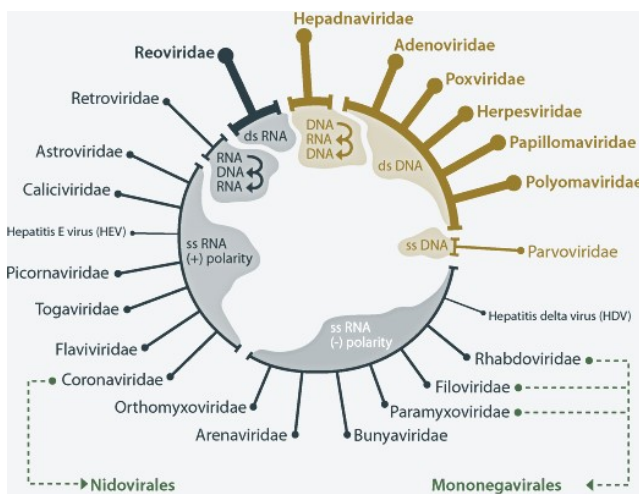
How they looks like?



<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 \approx 200-300 kB
- Genome may be segmented or non-segmented
- Linear or circular

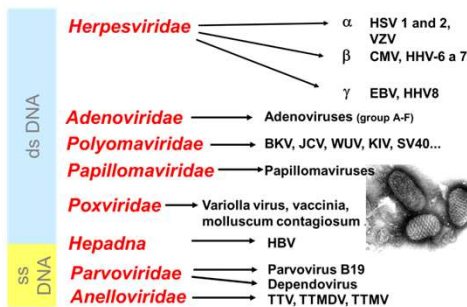
Does it make sense to know it?



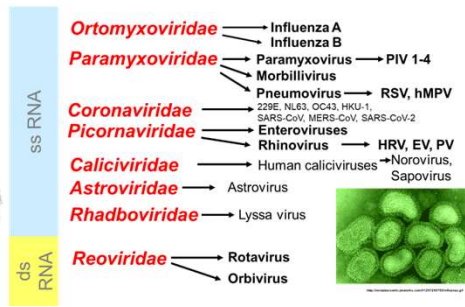
Many viruses around

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

DNA



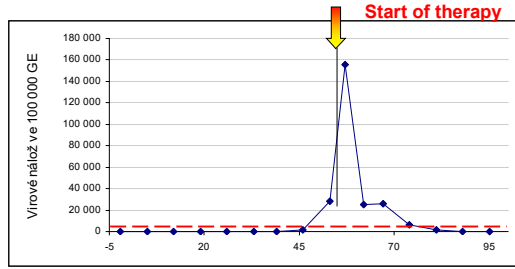
RNA



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

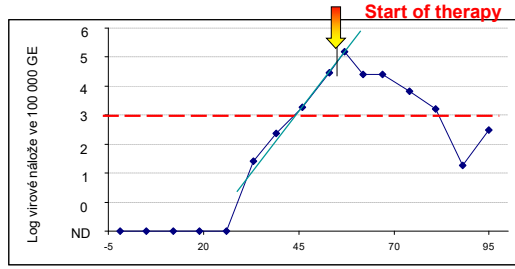
Smaller, less genes.
Rapid and acute disease.
Through TLR, IFN- bigger systemic impact of the infection.
Higher mutation rate.

Exponential virus proliferation



In vivo CMV doubling time

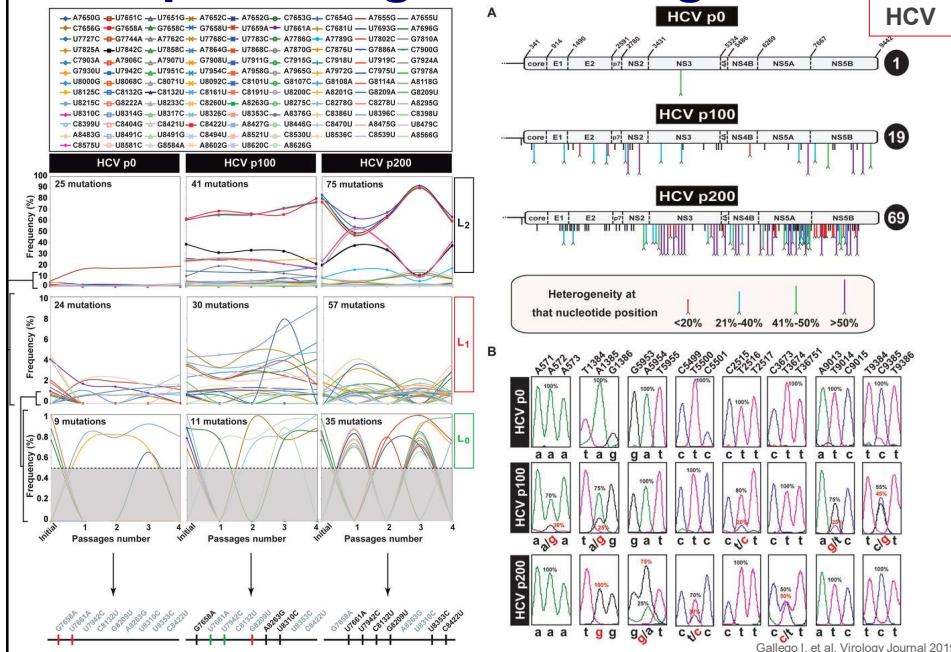
48-72 hours



Day	Diff. in days	CMV NVCs	Vypočítaná nálož do dalšího vyšetření při čase 48 hodin
19	7	0	
26	7	0	
33	7	260	2 060
39	6	2 300	27 500
46	7	18 700	224 400
53	7	281 700	

Patient with AML dg. after allo-HSCT

Rapid changes in viral genome



Gallego J. et al. Virology Journal 2019.

Rapid changes in CMV genome

Whole genome sequencing – NGS.

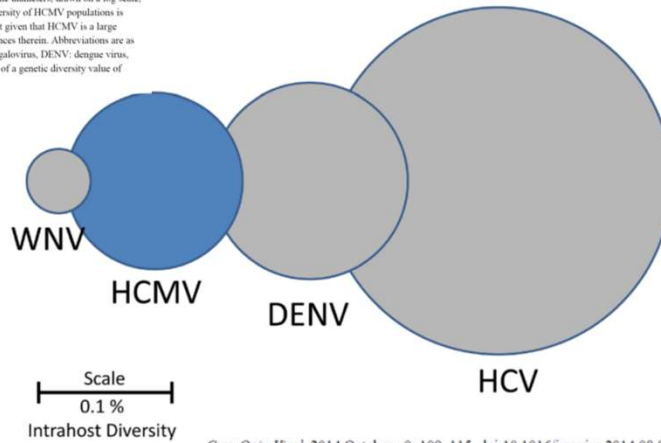
Human Cytomegalovirus Intra-host Evolution – A New Avenue for Understanding and Controlling Herpesvirus Infections

Nicholas Renzette¹, Laura Gibson², Jeffrey D. Jensen^{3,4,5}, and Timothy F. Kowalik^{1,6,*}

Figure 1. HCMV Intra-host genetic diversity as compared to RNA viruses
 Viral intra-host 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%.

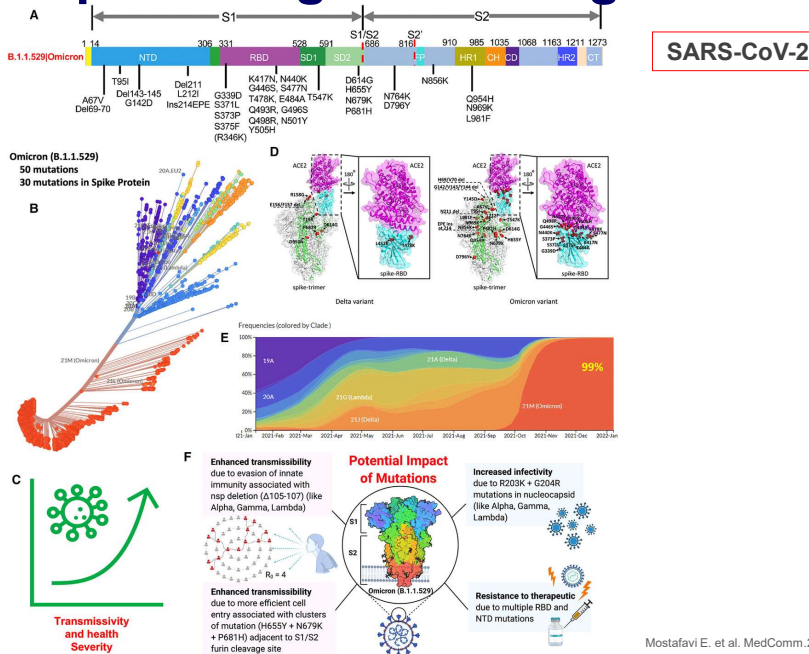
Re-infections in 10%

Predominant sequence in about 90% of viral population

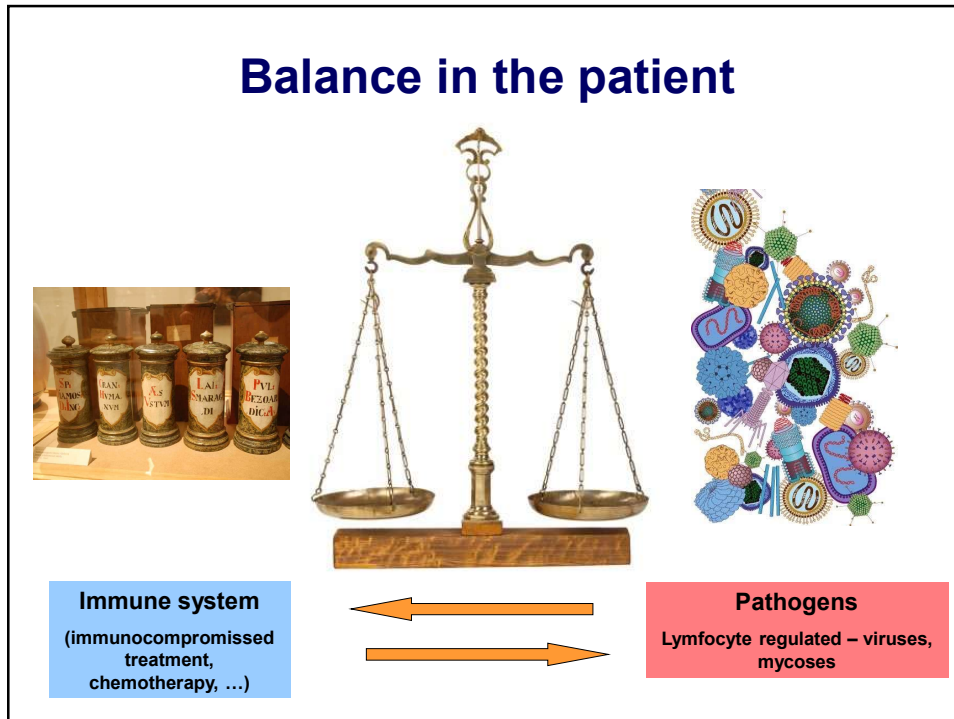


Curr Opin Virol. 2014 October ; 0: 109–115. doi:10.1016/j.coviro.2014.08.001

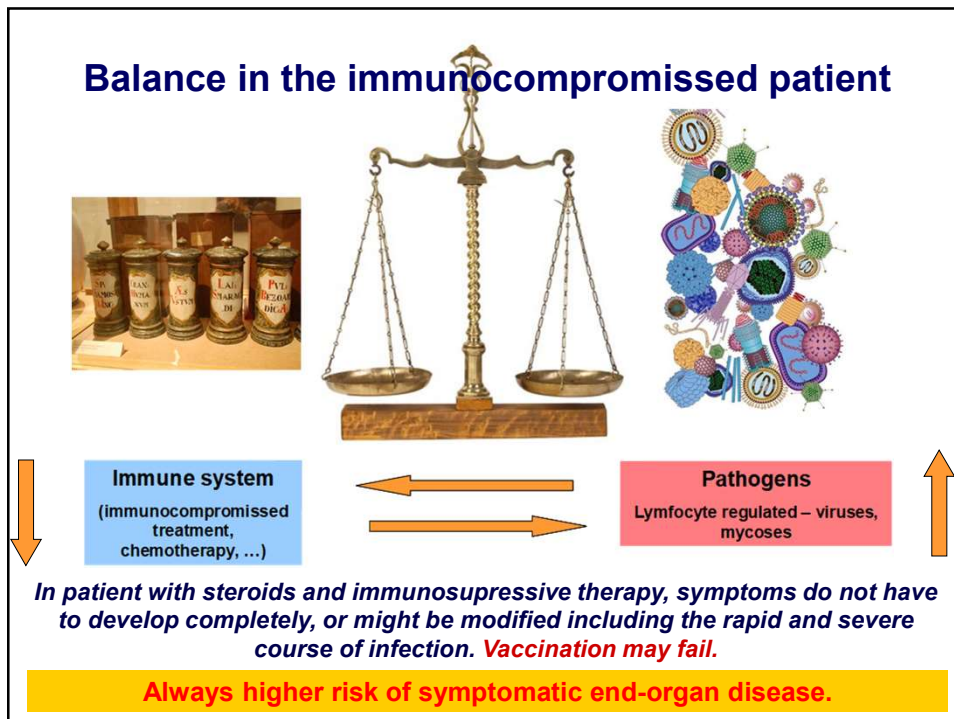
Rapid changes in viral genome



Balance in the patient



Balance in the immunocompromised patient



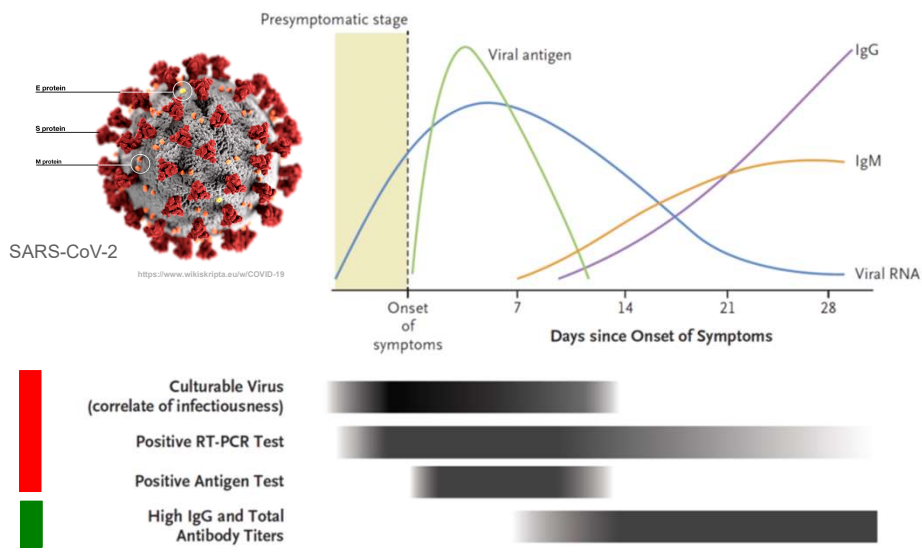
Detection methods in virology

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

- Detection of the antibodies
- (Signs of disease)

Indirect detection

Diagnostic window



Signs of the disease

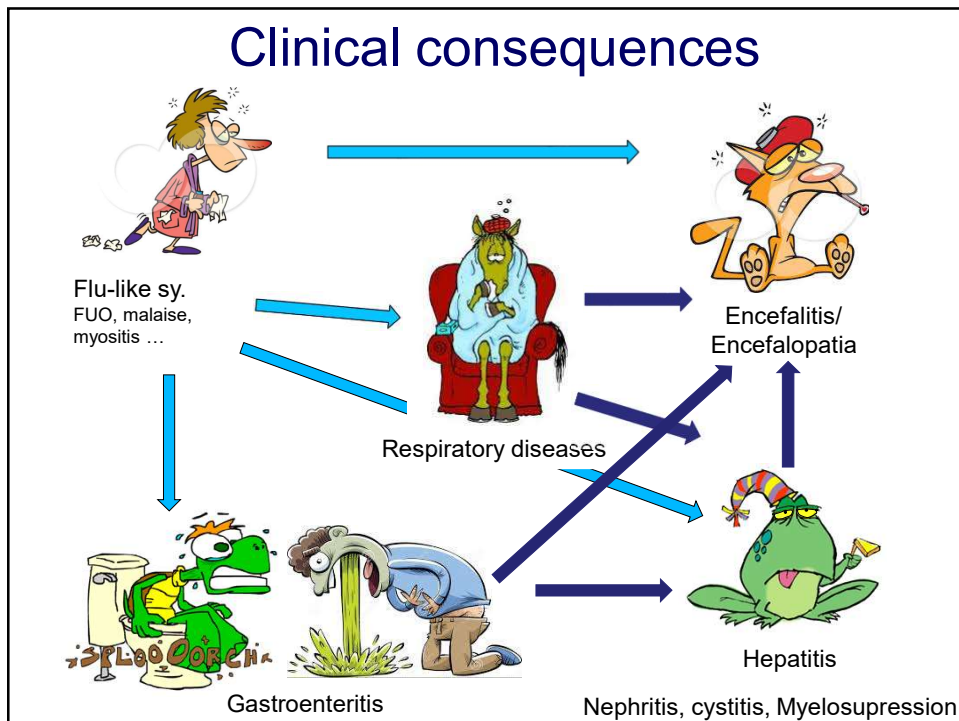
Clinical signs of disease leading to suspicion of viral infection (poliomyelitis) were described first 3 700 BC in Egypt.

Typical signs are e.g. in:

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



Clinical consequences

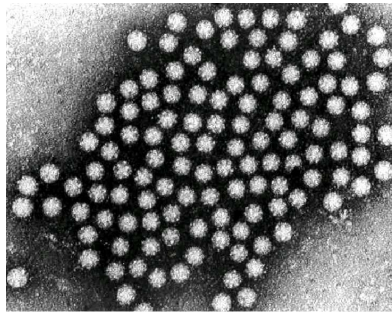


Astrovirus VA1/HMO-C: An Increasingly Recognized Neurotropic Pathogen in Immunocompromised Patients

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}

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

Neurotropic Pathogen HAsV VA1/HMO-C • CID 2015:60 (15 March) • 881



<http://www.casdc.ohio-state.edu/isa/lab/pictures/astro%20virus%2044.jpg>

Methods of the viral detection - DIRECT

Microscopic techniques

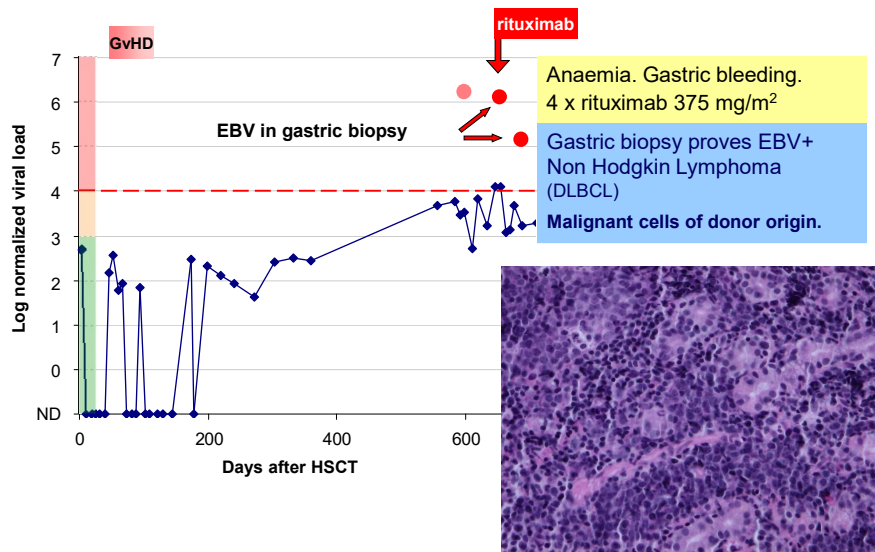
- **Electronmicroscopic detection of virus**
 - In liquid materials after virus concentration
 - In the tissues
 - Immunoelectron microscopic detection after signing of virus with specific antibody

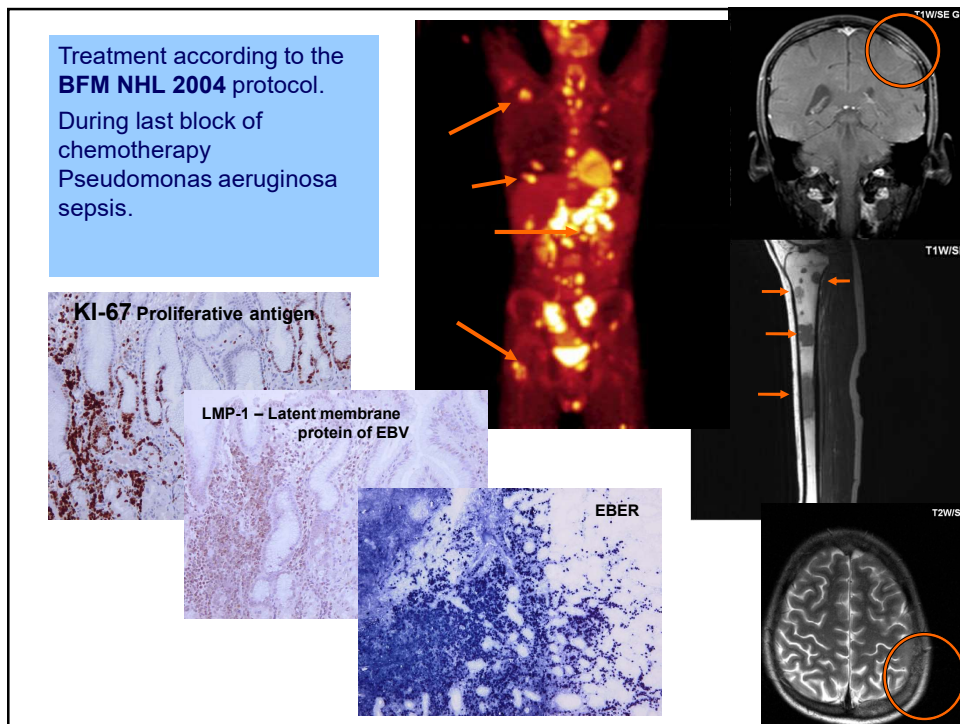
- **Immunohistochemical detection of virus in the cells**
 - Methods for histology testing of biopsies
 - Cytological technique

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)





Methods of the viral detection - DIRECT

Methods of cultivation

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

Tissue cultivation

Pros

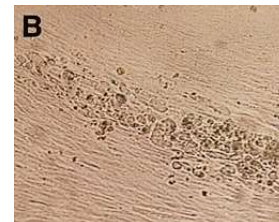
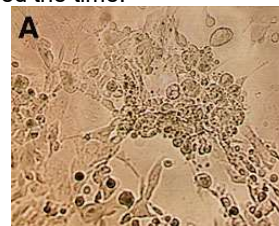
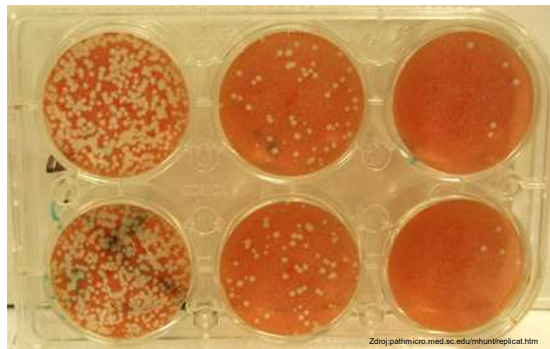
- Proving a „living“ virus
- Ability to do additional tests
- Detection wider spectrum of viruses

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

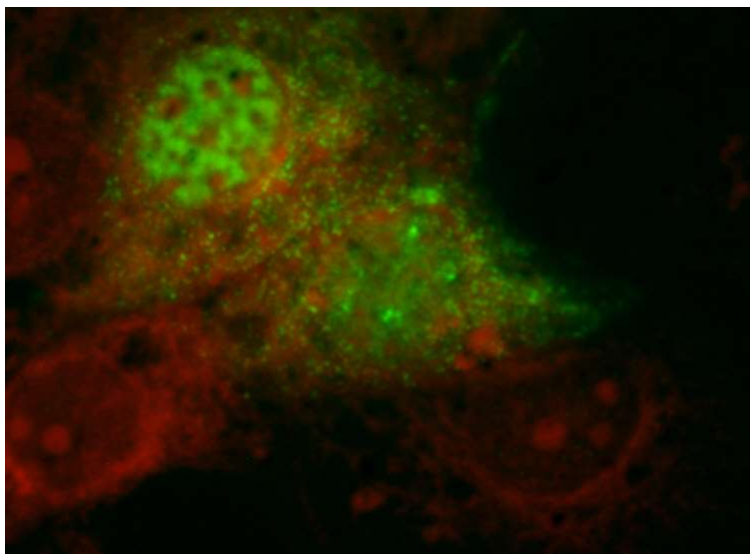
Additional possibility for viral detection is cultivation on tissue monolayers. J. Enders used it for the first time for poliovirus in 1949. Plaque forming assay was first used in 1952 by R. Dulbecco. Subsequently, plaque forming units (PFU) were established. Shell vial cultivation shortened the time.



Cytopathic effect - CMV

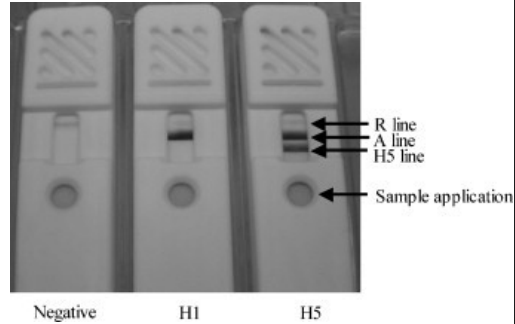


Influenza A virus on tissue monolayers - monoclonal antibody stained with FITC



Viral antigen detection

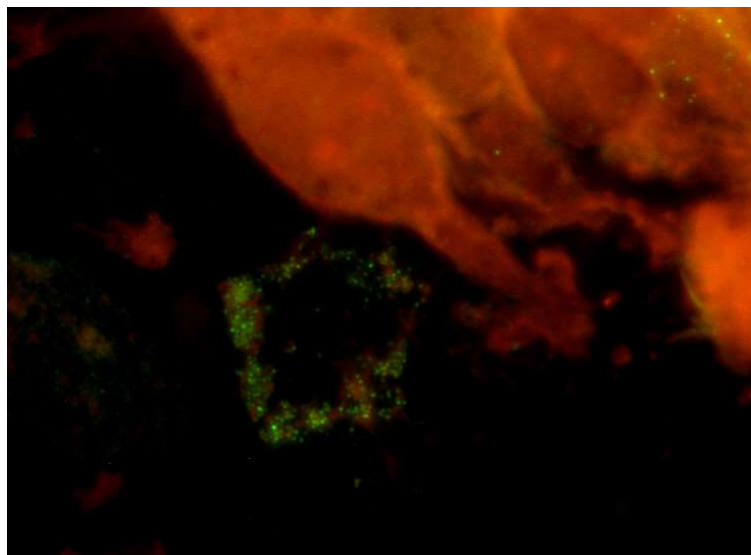
- Detection of single or only couple of pathogens
- Detection of presence/absence; sometimes possibility of (semi-) quantification.
- Based on antigen-antibody reaction



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

Price approx. 4-6 €

Antigen detection of Adenovirus in the lung tissue



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 immunocompromised host).
- Infection, in which is antigen present regularly in huge amounts (hepatitis B).

Is antigen detection easy?

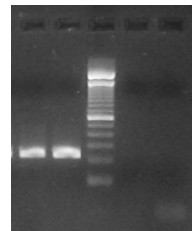
- Technically is antigen detection easy
- Difficult is interpretation of result
 - There is no „normal range“
 - Sensitivity of methods depend on type of used antigen – **no standardisation**
 - Difficult is interpretation of result

Nucleic acid detection

- Detection of one or couple agents 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 agents only
- Multiplex
 - Detection of more pathogens in a single reaction
 - Important is detection of product
- Quantitative
 - Competitive
 - Real - time PCR

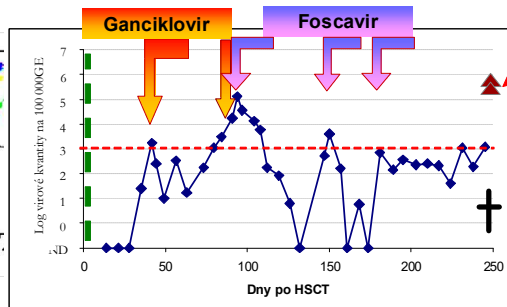
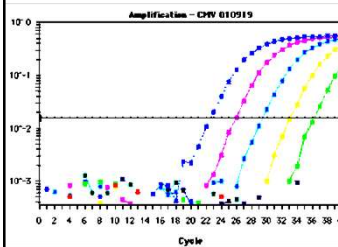


Using of PCR

- **Pros**
- High sensitivity
- rapid
- Highly specific
- Possibility of quantification
- **Cons**
- Sensitive for manipulation
- Detection of vial and non-vial agents
- Risk of inhibition and false positivity

Using of Real-time PCR

- Quantification of microbial agents to find diagnosis and prognosis
- Monitoring of patients in immunosuppression (quick start of the treatment)
- Monitoring of virostatic treatment (detection of resistance in patient – non-responders)

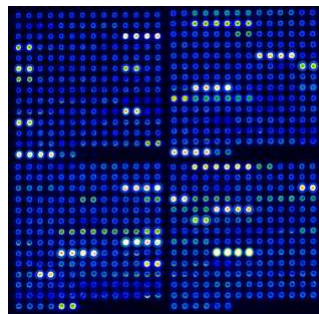


Sequencing and detection of agent according to the database

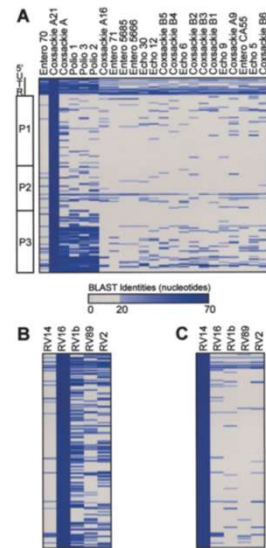
- Detection of nucleotid sequence according to the database
- Less useful in virology
- Quality of database matters
- At the present time more supplementary

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 agents - sensitive
 - PCR depends on type of techniques (primers, multiplex...)
- Cultivation is easily influenced by experience and type of agents (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

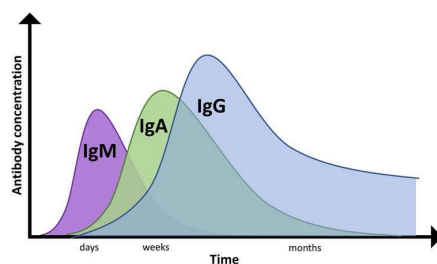
Comparing of techniques – possibility of wide detection

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

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

Detection of antibodies

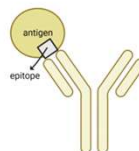
- Can detect immunoglobuline in different classes (IgG, IgM, IgA)
- At the beginning of the infection, there is no specific antibody production
- Not suitable for monitoring of the treatment



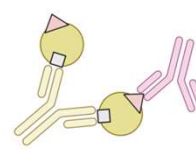
<https://www.raybiotech.com/learning-center/covid-19-biology-101/>

Affinity vs. Avidity

Affinity - strength of a single interaction



Avidity - total strength of a multivalent interaction



<https://www.rapidriver.com/wp-content/uploads/2020/01/2-Affinity-vs.-Avidity-e1673020820106-768x438.png>

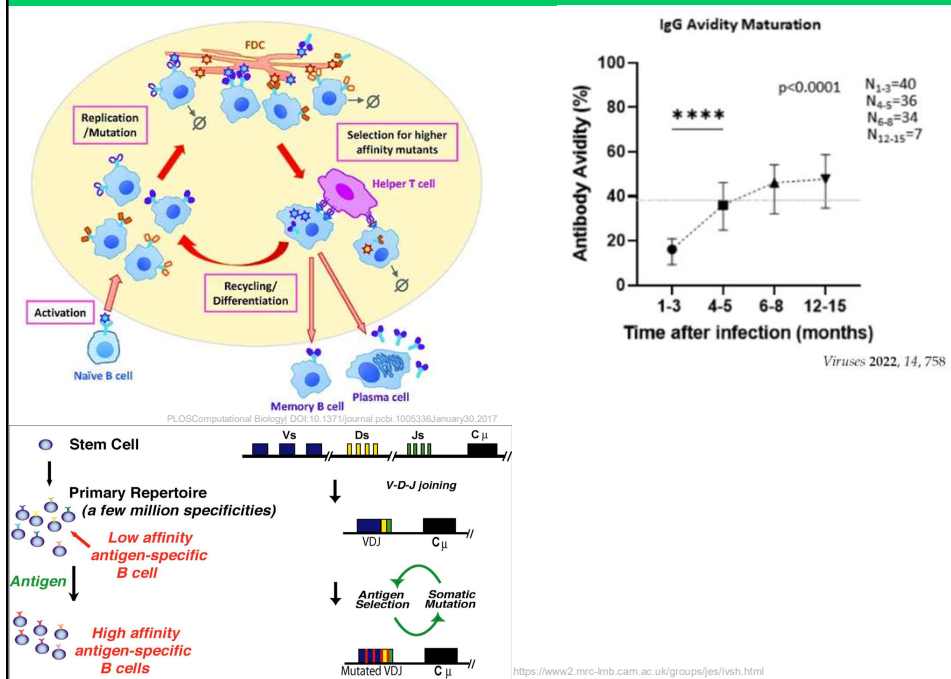
Main usage of the antibody detection

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

Limited or small impact of antibody detection

- **Infections with intracellular bacterias** (Mycobacterium tuberculosis)
- **Local infections** (uncomplicated salmonellosis, tonsillitis, urinary tract infections...)
- **Reactivation of persistent infections** (herpes)
- **Detection of infections in the immunocompromised host**

Methods of the viral detection - INDIRECT



Methods of the viral detection - INDIRECT

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.

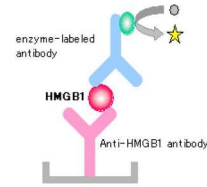
Immunochemical methods

EIA (enzyme immunoanalysis)

IF (immunofluorescence)

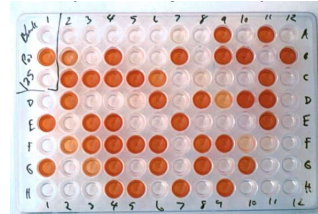
RIA (radio-immunoanalysis)

ELISA (enzyme-linked immunosorbent assay)



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

Disadvantage: more expensive, sometime unspecific results



Why can antibody detection fail?

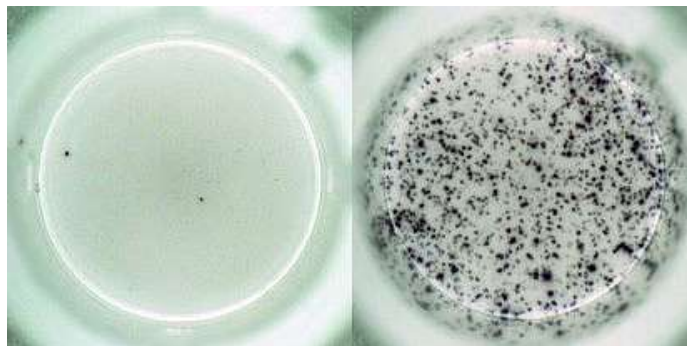
- Significant part of infection is destroyed by unspecific immunity
(no activation of specific immunity)
- Detection has limited sensitivity, method is inappropriate or blood sample has been drawn too early
- Infection was caused by another than tested pathogen.

Is antibody detection really easy?

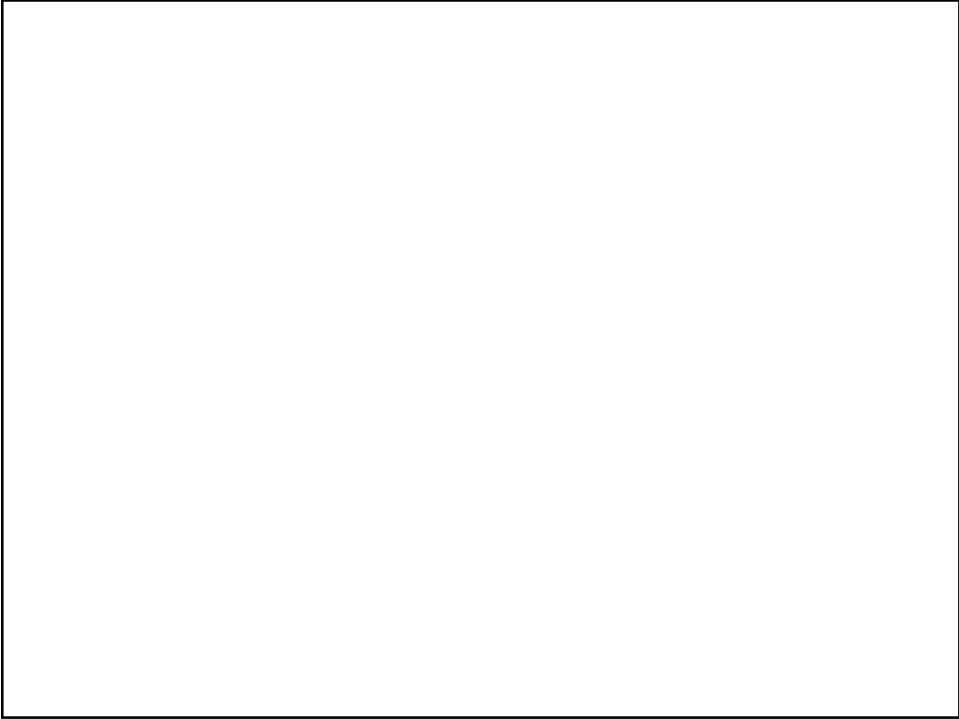
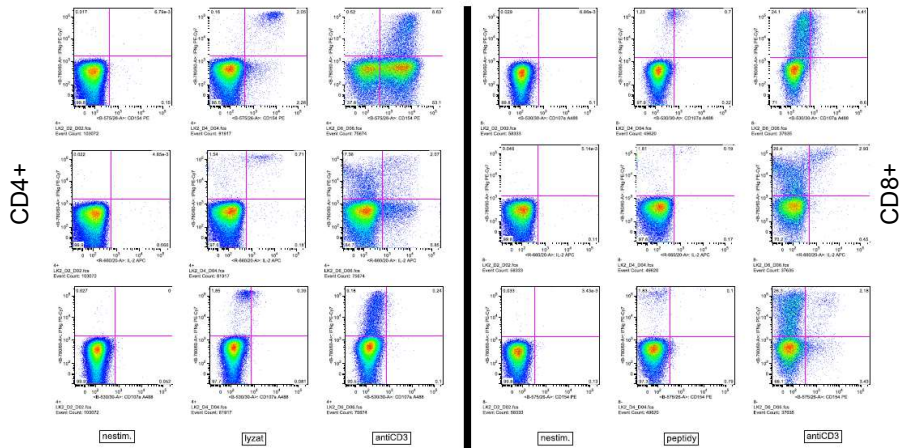
- Technically is antigen detection easy
- Difficult is interpretation of result
 - There is no „normal range“
 - Sensitivity of methods depend on type of used antigen – no standardisation
 - Difficult is interpretation of result
- The immune system of every person works a bit different

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.



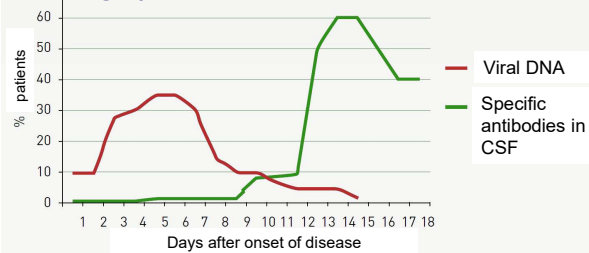
Detection of specific lymphocytes



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 knowledge 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 length of transport (up to 24 hours)
- **Antigen detection**
 - It is important to avoid sample destruction
- **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

- Standard result in a reasonable time
- Express testing in severe clinical situations
- Consultation of diagnostic possibilities
- Interpretation of the result and advise with further steps in diagnostics and therapy
- Inform clinical staff about diagnostic 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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