
بسم الله الرحمن الرحيم

Laboratory Diagnosis of Viral Meningitis

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Central Nervous System Infections

- **Meningitis**
  - Inflammation of the meninges
  - Infectious agents include: bacteria, viruses, fungi and protozoa.
- **Encephalitis**
  - Inflammation of the brain itself
  - Caused by many types of organisms
- **Myelitis**
  - Inflammation of the spinal cord (VZV, CMV, EBV)

Meningitis classified

- **Acute pyogenic meningitis**

  usually bacterial meningitis

- **Aseptic meningitis**

  Aseptic meningitis is a syndrome of multiple etiologies, but most cases are caused by a viral agent.

- **Chronic meningitis**

  Chronic meningitis is defined as persistence of meningeal inflammation for over 4 weeks.Mumps and lymphocytic choriomeningitis (LCM) virus infections occasionally persist for 3 to 4 weeks but true chronic viral meningitis is rare except in patients who are immunodeficient.

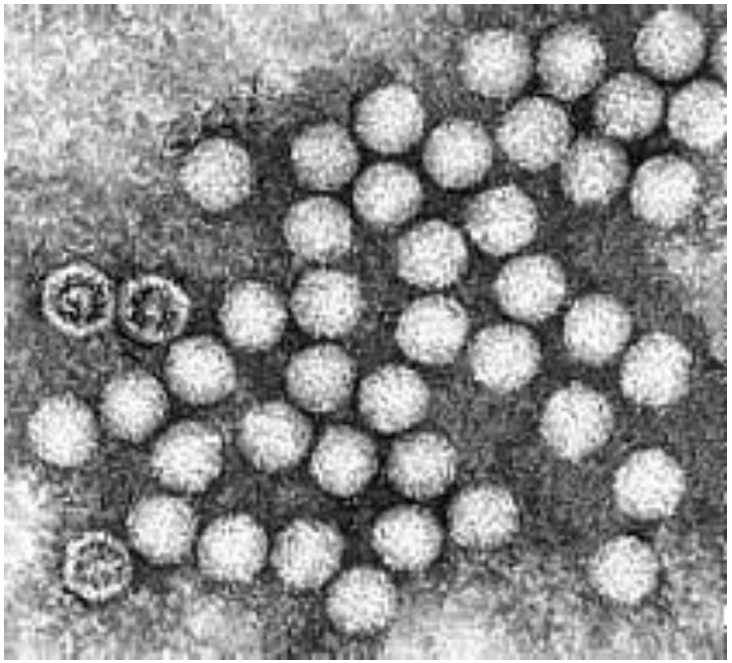
Viral Meningitis (Aseptic Meningitis)

❖ Etiological Agents:

- ➢ Enteroviruses, most common (Coxsackie and Echovirus)
- ➢ Herpes Simplex virus
- ➢ Lymphocytic Choriomeningitis virus (LCM)
- ➢ Mumps
- ➢ Varicella Zoster virus
- ➢ Measles virus
- ➢ Arbovirus
- ➢ HIV

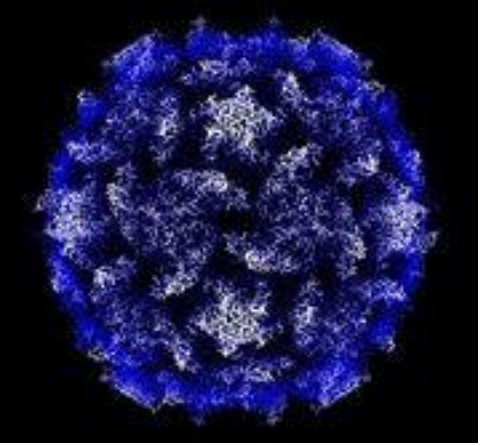
Other less common causes include:

EBV, CMV, and adenoviruses



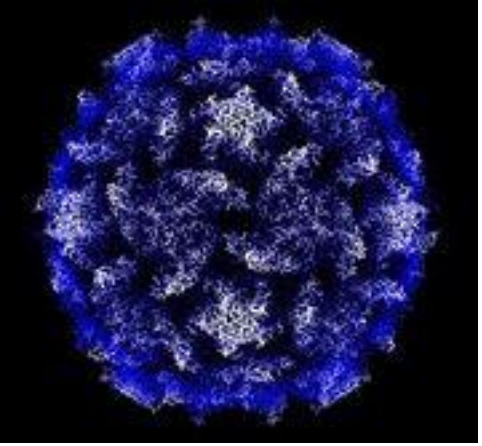
Classification of Picornaviridae

- ***Enterovirus*** **(enteroviruses)**
  - **a) Polioviruses types 1, 2 and 3**
  - **b) Coxsackieviruses**
  - **c) Echoviruses**
  - **d) Enteroviruses**
- ***Rhinovirus*** **(rhinoviruses)**
- ***Hepatovirus*** **(hepatitis A virus)**
- ***Parechovirus*** **(parechoviruses)**
- ***Aphthovirus*** **(foot-and-mouth disease viruses)**
- ***Cardiovirus*** **(cardioviruses)**



Enteroviral Meningitis

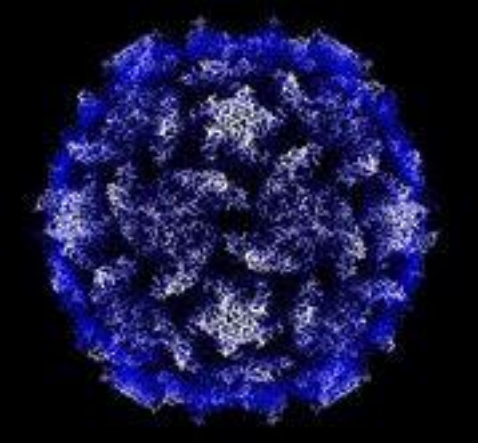
- Enterovirus infections account for most cases of viral meningitis.
- Are a diverse group of RNA viruses including Coxsackie A & B, Echoviruses, and polioviruses.
- Coxsackie A7, A9, B1-6, echovirus 4, 6, 11, 14, 16, 25, 30, 31 are commonly involved.
- Transmitted primarily by fecal-oral route, but can also be spread by contact with infected respiratory secretions. The incidence is increased in the summer months, but cases occur throughout the year.
- During outbreaks most cases occur in children under 5 years of age.



Enteroviral Meningitis

The human parechoviruses 1 and 2, formerly echovirus 22 and 23, are more commonly associated with diarrhea and respiratory illness than with CNS disease.

Cases of meningitis and encephalitis occasionally occur with these agents.



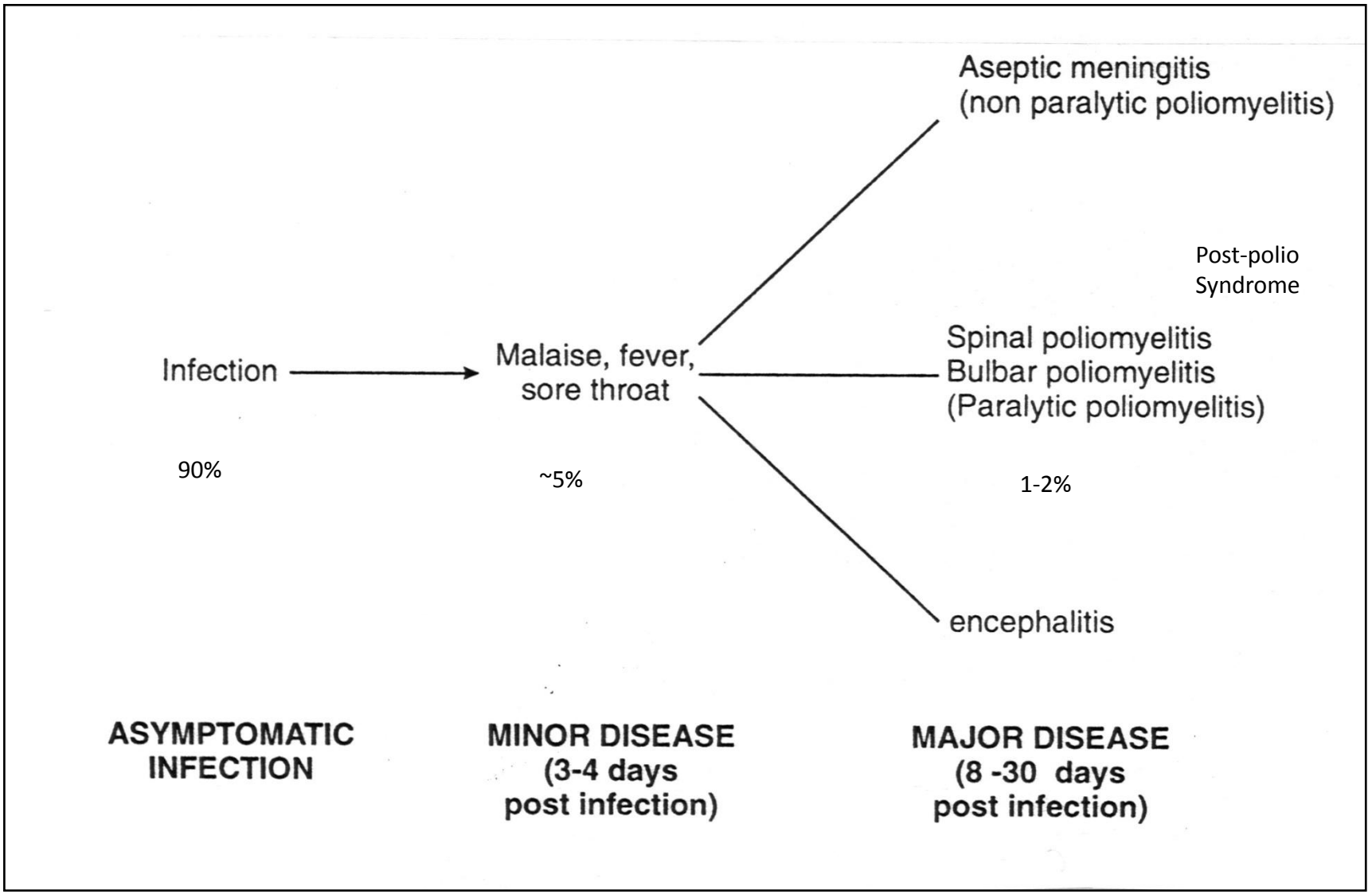
Poliovirus - Pathogenesis

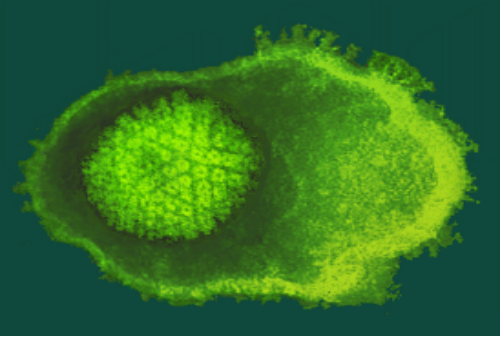
- initial virus replication is in lymphoid tissues of tonsils and pharynx
  - virus is swallowed (resists acid) and replicates in the lymphoid cells of the Peyers patches
    - primary viremia takes the viruses to CNS, anterior horn cells and brain motor cortex - producing paralysis of the extremities
      - virus may cross the blood brain barrier into CNS
      - or virus may move via peripheral nerves to the CNS
  - if virus spreads to other areas of the CNS, like medulla and cranial nerve, then bulbar paralysis of respiration, pharynx, etc
    - if virus is shed back to the blood from the CNS, this is secondary viremia
  - pathogenically polio viruses are neurotrophic
  - humoral antibody is required for recovery and prevention

Clinical Presentations of Poliovirus Infection

- **Approx. 90% of infections are ASYMPTOMATIC**
- **Minor illness in 4-8% of low-grade fever, sore throat**
- **Aseptic Meningitis in 1-5%**
- **Asymmetrical acute flaccid paralysis with areflexia of limbs involved in 0.1%-2% of infections (Respiratory Muscles may be involved)**
- **Residual Paralytic Disease in 2/3 of these**

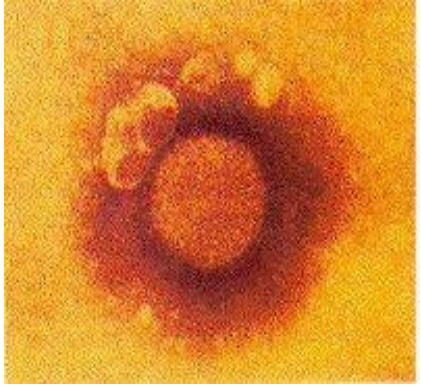
Idealized Scheme of the Course of Infection with Poliovirus





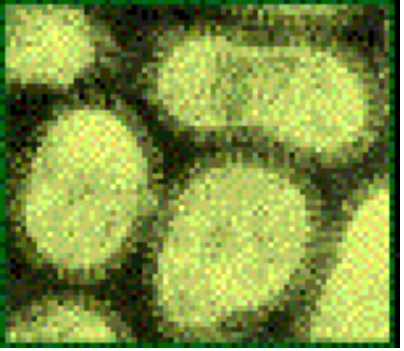
Herpes Simplex Meningitis

- Generally caused by HSV-2 (as opposed to encephalitis which is caused by HSV-1)
- dsDNA virus
- Can be due to primary or recurrent HSV infection
- The genital lesions are typically present (85% of the time), and usually precede the CNS symptoms by seven days.
- HSV meningitis can be recurrent, these patients may not have clinically evident genital lesions.



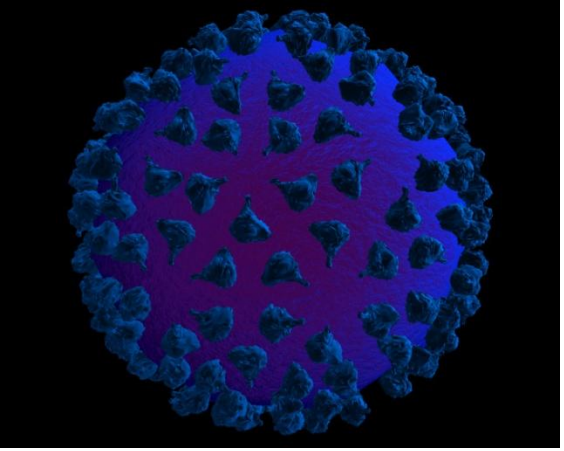
Lymphocytic Choriomeningitis Virus

- ssRNA virus of the arenavirus group
- LCM is excreted in the urine and feces of rodents, including mice, rats, and hamsters.
- It is transmitted to humans by either direct contact with infected animals or environmental surfaces.
- Infection occurs more commonly in the winter months.
- Symptoms generally include a influenza like illness.



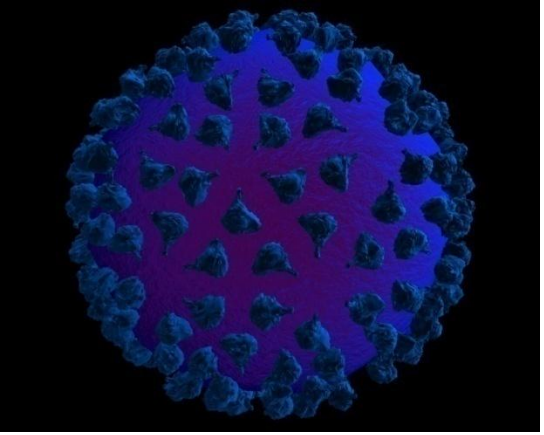
Mumps Meningitis

- Caused by paramyxovirus which is a ssRNA virus
- Prior to the creation of the mumps vaccine in 1967, it accounted for 10-20% of all cases of viral meningitis.
- Even now this virus causes a significant minority of cases in unvaccinated adolescents and adults.
- In patients who do acquire mumps, CNS infection occurs rather frequently, with CSF pleocytosis detected in 40-60% of patients, and 10-30% of those have clinical signs and symptoms of meningitis.



HUMAN IMMUNODEFICIENCY VIRUS

Direct HIV infection of the CNS occurs in 10 to 50% of patients with AIDS, ultimately producing dementia. Multinucleated giant cells are the pathognomonic feature in deep grey and central white matter. HIV encephalitis may be more common in drug users than in HIV-infected homosexuals.



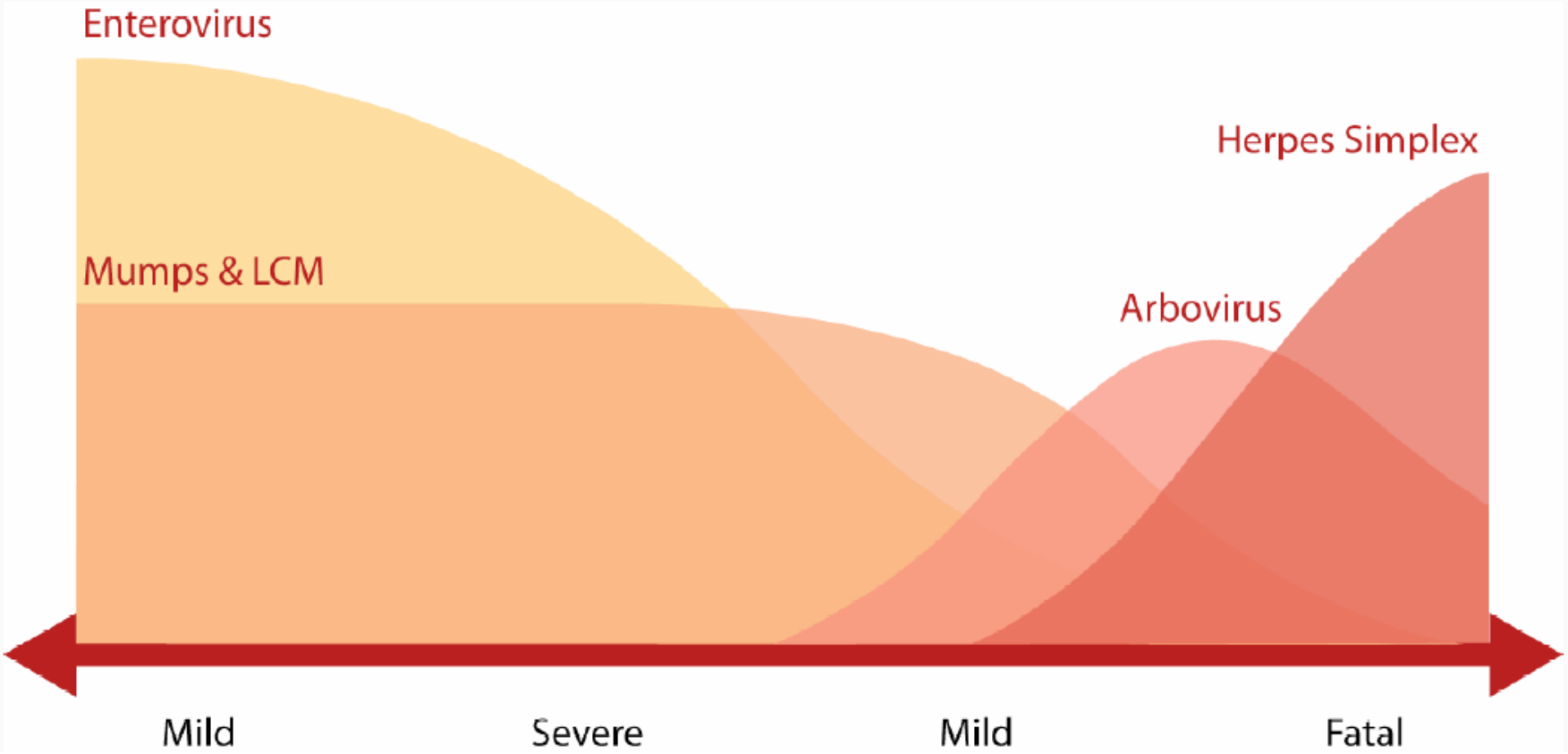
HUMAN IMMUNODEFICIENCY VIRUS

The incidence of HIV encephalitis, which had fallen with more effective therapy, has risen in recent years perhaps due to longer survival and greater exposure of brain to HIV. HIV lymphocytic meningitis and HIV encephalitis can be found at various stages of HIV infection and tend to develop before opportunistic infections.

Miscellaneous viruses

- West Nile Virus,
- St Louis Encephalitis,
- California Encephalitis,
- VZV,
- EBV,
- CMV,
- adenoviruses
- Less common causes of meningitis, but they do occur.
- In most cases the course is self-limited, and the treatment is supportive in nature.

Viruses and Severity of Disease



Meningitis **Encephalitis**

Laboratory Diagnosis

Rapid recognition of acute meningitis is vital, and the differentiation between bacterial, partly treated bacterial and viral meningitis is critical to allow appropriate treatment of the infected individual and management of contacts.

SAFETY CONSIDERATIONS

Containment Level 2 unless infection with a Hazard group 3 & 4 organism is suspected, in which case work should be performed in a microbiological safety cabinet under containment level 3 conditions.

Classification of viral agents of CNS disease

Hazard Group 2	Hazard Group 3	Hazard Group 4
Enteroviruses	LCM	Nipah
Herpesviruses including Herpes Simplex virus, Varicella Zoster virus, Epstein Barr Virus, Cytomegalovirus	Flaviruses including dengue, Japanese encephalitis, Murray Valley encephalitis, St Louis encephalitis, West Nile fever	
Mumps	Herpes simiae	Russian Spring-Summer encephalitis
Measles virus	HIV	Omsk haemorrhagic fever
Adenoviruses	Rabies	
	Alphaviruses including Eastern equine encephalitis, Western equine encephalitis, Venezuelan encephalitis	
	Tickborne encephalitis, louping ill	

Types of specimen:

- Cerebrospinal fluid
- serum
- plasma
- brain biopsy
- swabs
- feces
- urine

SPECIMEN COLLECTION TRANSPORT AND STORAGE

➢OPTIMAL TIME OF COLLECTION

Preferably before antimicrobial therapy is started, but this must not be delayed unnecessarily pending lumbar puncture and CSF culture.

Specimens should be transported and processed as soon as possible.

SPECIMEN COLLECTION TRANSPORT AND STORAGE

➢SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Specimens should be cultured as soon as possible after receipt, ideally within 10 minutes and within a maximum of two hours.

Cells disintegrate and a delay may produce a cell count that does not reflect the clinical situation of the patient.

Do not refrigerate specimen until after microscopy and bacterial culture have been performed.

The specimen should then be refrigerated pending further investigation.

SPECIMEN COLLECTION TRANSPORT AND STORAGE

CEREBROSPINAL FLUID

- CSF should be collected by lumbar tap into sterile containers. It is essential that CSF is sent for cell count, bacteriology, biochemistry and virology.
- CSF may be stored at +4°C if delays in processing for virus culture or viral PCR will be less than 24 hours.
- If greater delays are likely CSF should be frozen at –80°C, while clotted blood samples should be separated and the serum stored at –20°C.

SPECIMEN COLLECTION TRANSPORT AND STORAGE

➢SERUM

Clotted blood 7 to 10 mL, should be collected by venepuncture for serology, particularly IgM assays. Serum can also be used for enterovirus PCR.

➢ PLASMA

EDTA-anticoagulated blood 5mL should be collected into sterile tubes by venepuncture.EDTA-anticoagulated whole blood is suitable for PCR assays, especially herpesvirus assays, eg HSV in neonatal CNS disease, CMV and EBV in patients who are immunocompromised.

SPECIMEN COLLECTION TRANSPORT AND STORAGE

BRAIN BIOPSY

- Brain specimens should be collected unfixed into a sterile container.
- Brain smears can be used for viral antigen detection by immunofluorescent antibody staining, and for electron microscopy with negative staining.
- Emulsified brain tissue is suitable for tissue culture and after proteinase K treatment for PCR.

SPECIMEN COLLECTION TRANSPORT AND STORAGE

➢SWABS

Swabs when taken should be put into virus transport medium. Suitable swabs include throat swabs for a range of virus cultures and PCR.

➢FAECES

Faeces should be collected for enterovirus culture into clean containers.

➢URINE

Ten to 20 mL of urine should be collected into sterile containers (without preservatives) for mumps virus culture and mumps PCR.

Examination of CSF

The diagnosis of meningitis from the examination of CSF involves the following :

- **Complete cell count**
- **Differential leukocyte count**
- **Examination of Gram-stained smear**
- **Culture**
- **Determination of glucose and protein concentrations**
- **PCR**
- **Antigen testing**

SPECIMEN PROCESSING

➢TEST SELECTION

Divide specimen, if multiple samples are not taken after performing microscopy and bacterial culture, for appropriate procedures

An unopened sample, if available, is preferred for PCR.

➢APPEARANCE

Describe turbidity and whether a clot is present.

Describe colour of supernatant after centrifugation.

SPECIMEN PROCESSING

- **MICROSCOPY**
  - **Standard total cell count**

Perform total WBC and RBC counts on the uncentrifuged specimen, preferably the last specimen taken, in a counting chamber.

Cell counts should not be performed on specimens containing a clot (which would invalidate the result).

- **Differential leukocyte count**
- **CULTURE AND OTHER INVESTIGATION**

Normal CSF values

Leukocytes	Neonates 1-4yr old 5yr-puberty Adults	0 – 30 cells/mm^3 0 - 20 cells/mm^3 0 - 10 cells/mm^3 0 - 5 cells/mm^3
Erythrocytes	Newborn Adults	0 - 675 cells/mm^3 0 - 10 cells/mm^3
Protein	Neonates ≤6d Others	0.7 g/L (70 mg/dL) 0.2 - 0.4 g/L (20 - 40 mg/dL) (<1% of serum protein concentration)
Glucose		≥60% of simultaneously determined plasma concentration (CSF: serum ratio ≥0.6)

CSF Findings in Infants and Children

Component	Normal Children	Normal Newborn	Bacterial Meningitis	Viral Meningitis
WBC (cells/mm^3)	0-6	0-30	100 to 10,000	20-500
Neutrophils (%)	0	2-3	>80	< 50
Glucose (mg/dL)	40-80 0.6 CSF:serum	32-121	<40 <0.4 CSF:serum	>50
Protein (mg/dL)	20-30	19-149	>50	50-100
Erythrocytes/ mcL	0-2	0-2	0	0-2

Viral Culture

- **Because viruses require cellular machinery for replication, living systems must be used.**
- **Isolation of viruses on tissue culture from CSF, blood or urine is the gold standard for diagnosing many viral pathogens causing meningitis**

Viral Culture

Advantages:

- **is an amplification method that increases the amount of the pathogen, facilitating detection and characterization.**

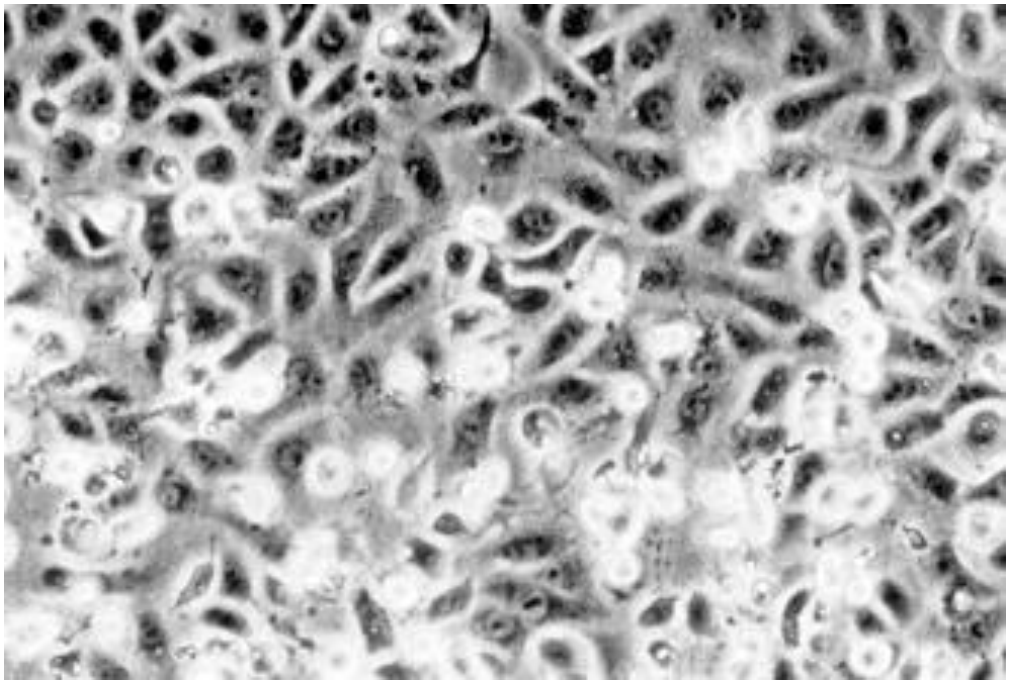
- **provides an isolate of viable virus that and can be stored for future studies.**

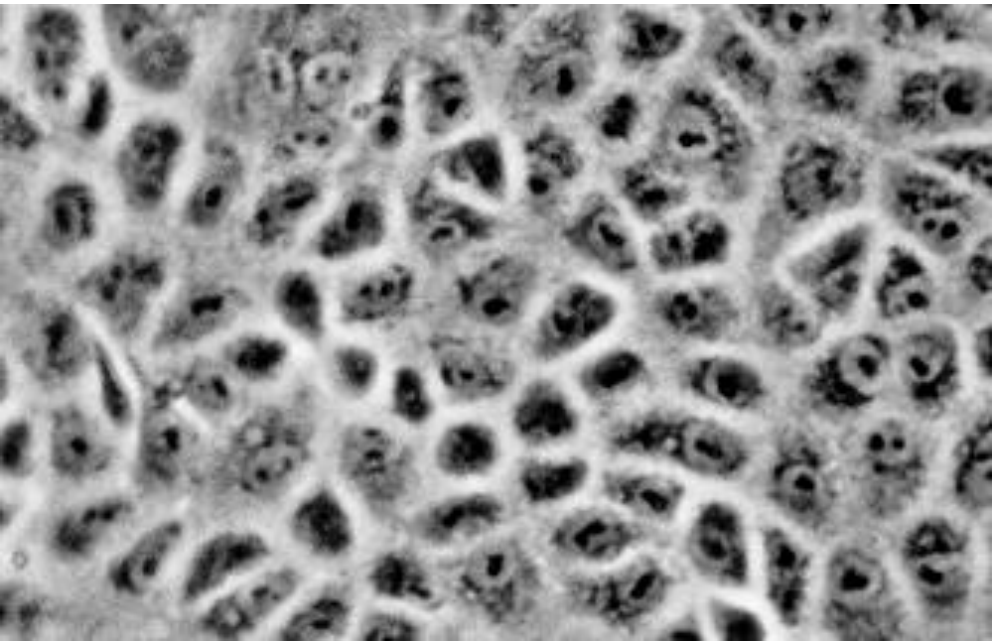
Viral Culture

Disadvantages:

- **including requirement for specialized facilities and expertise, expense, relatively prolonged time to detection, and the relatively limited range of viruses that can be detected.**
- **Viral culture requires more attention to conditions of transport than specimens submitted for detection of viral antigens or nucleic acids, because the viability of the virus must be preserved.**

Infected monkey cells





Non-infected monkey cells

Syncytium and CPE

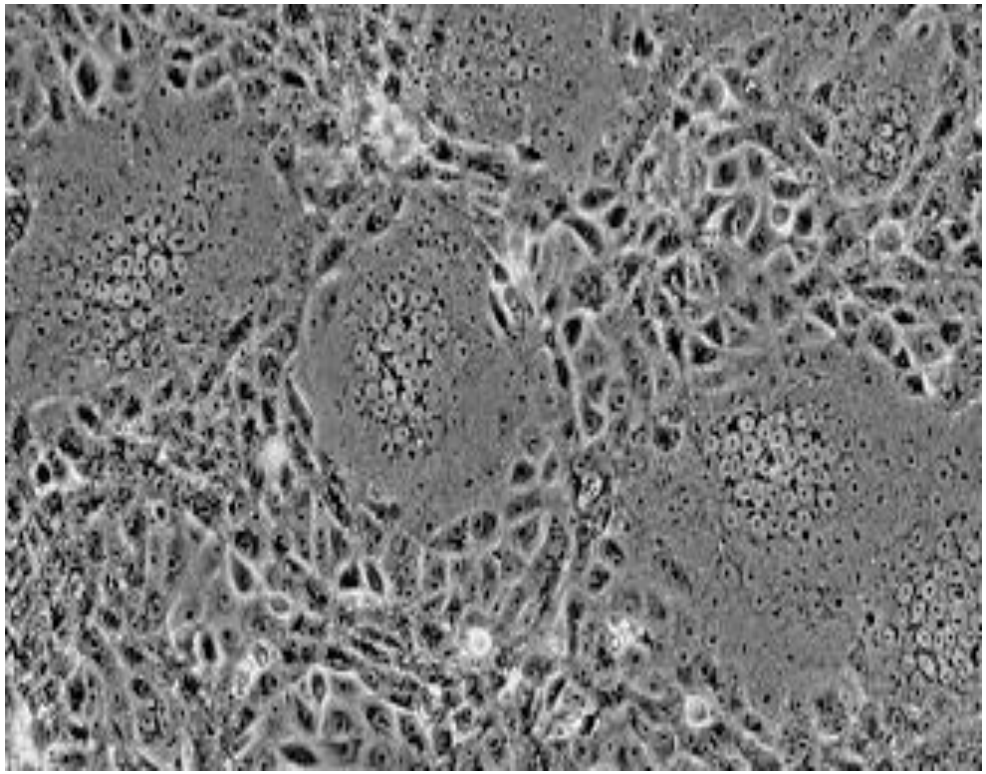
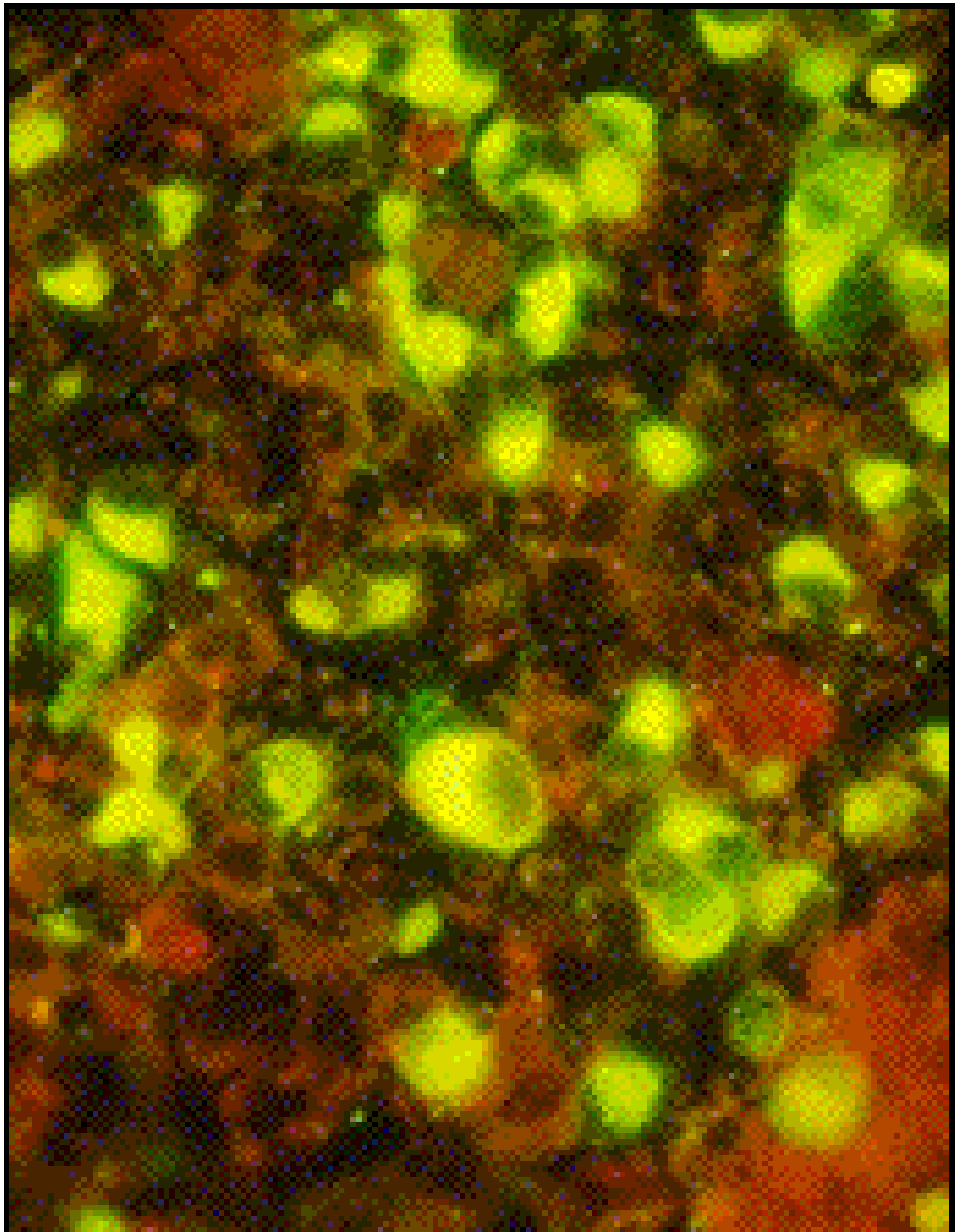


Fig. 1, Cytopathic effects of enterovirus 71 in rhesus monkey kidney cells

IF Test



Serologic Assays

Cerebrospinal Fluid Serology:

- **Serologic testing can be applied to CSF for the diagnosis of central nervous system (CNS) infection.**

Serologic Assays

Cerebrospinal Fluid Serology:

- **For the diagnosis of encephalitis caused by the alphaviruses, bunyaviruses, or flaviviruses, the presence of virus-specific antibodies in CSF is highly suspicious and the presence of virus-specific IgM antibodies in CSF is diagnostic.**

Cerebrospinal Fluid Serology

- **common viruses (e.g., herpesviruses) or respiratory viruses, the mere presence of virus-specific antibodies in CSF is not diagnostic of CNS infection because antibodies produced in the blood are present in the CSF even in the absence of CNS infection.**

Cerebrospinal Fluid Serology

- **Intrathecal synthesis of a specific antiviral antibody is evaluated by determining the quotient of two ratios:**
- **the ratio of specific antiviral antibody level in CSF to the level in serum,**
- **the ratio of total IgG in CSF to total IgG in serum**
- **A quotient greater than 1.5 is evidence of intrathecal antibody synthesis of the specific antibody.**

Multiplex PCR

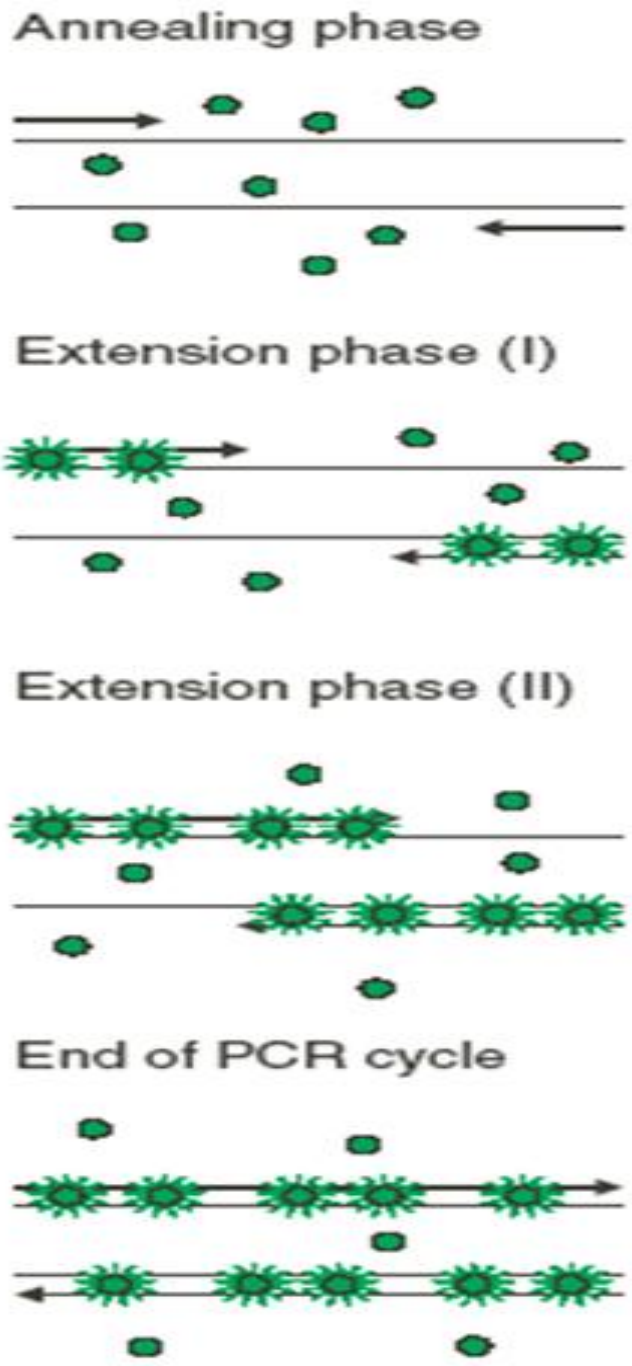
- **In multiplex PCR more than one target sequence can be amplified by including more than one pair of primers in the reaction.**
- **Design Primers Separately**

Real-time PCR

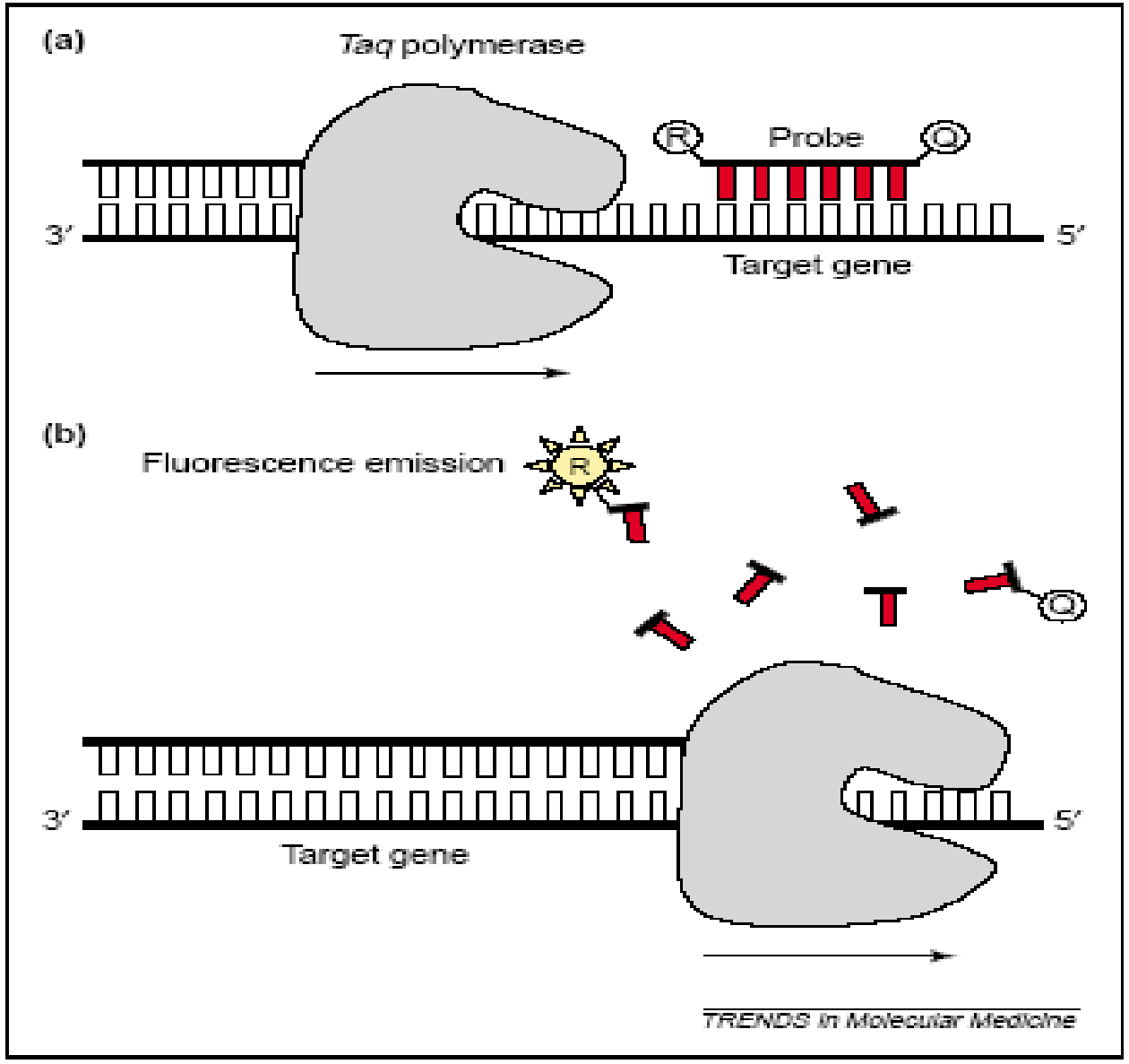
- **advantages**
- **The chance for contamination decreased, cause the systems are closed.**
- **Rapid cycling times (1 hour).**
- **Of great importance, quantitation of PCR targets.**
- **Very sensitive**

DNA-binding agents

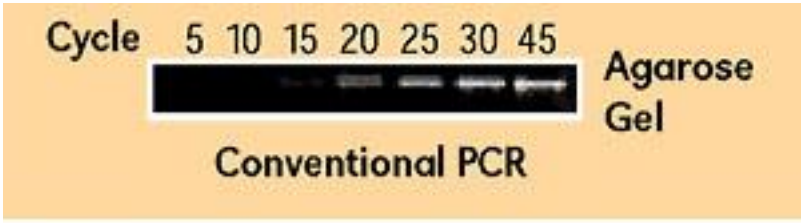
(SYBR Green)

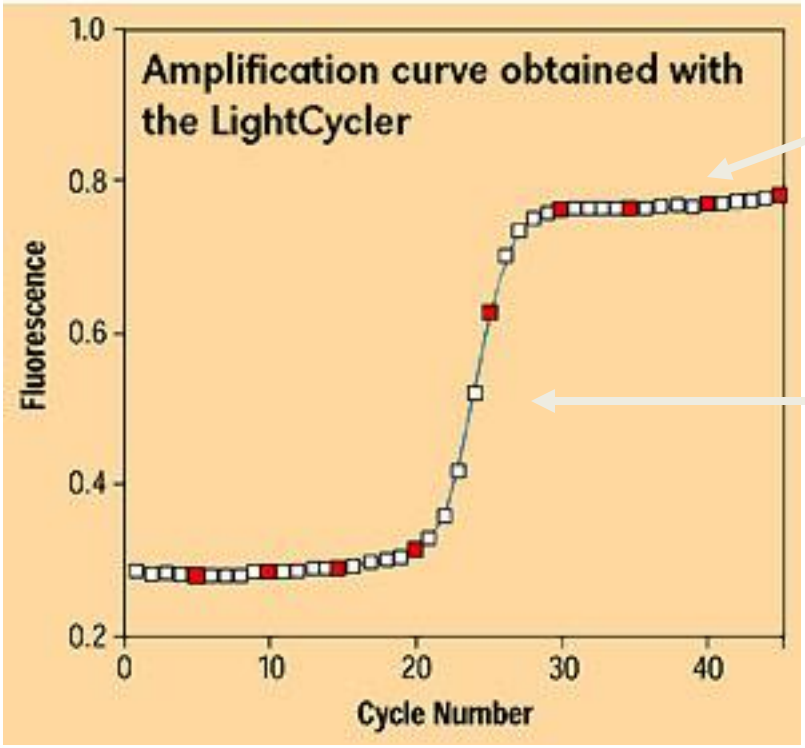


Hydrolysis probes (TaqMan)



Fluorescence Monitoring





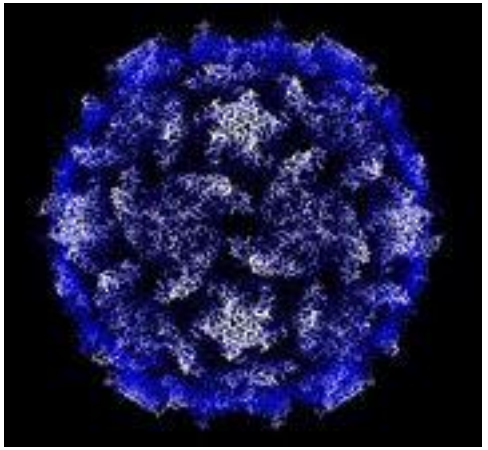
Plateau:
Qualitative
end-point read

Exponential:
Quantitative
real-time read

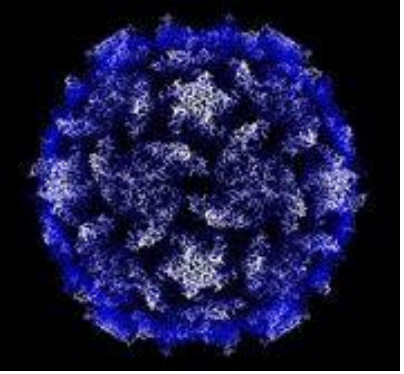
Diagnostic assays for central nervous system viral infections

No.	Organism	Specimen(s)	Suggested methodology	Comments
1.	*Herpesviridae* (HSV 1 and 2, CMV, VZV, EBV, HHV 6)	CSF and paired serum specimens	Isolation using cell culture, antigen detection and PCR, antibody detection	PCR has replaced culture as the "Gold standard"
2.	*Enteroviruses*	CSF and paired serum specimens	Isolation using cell culture, detection of rising antibody titres and PCR	PCR using genus-specific conserved primers has proved most sensitive
3.	*Flaviviridae* (JEV, West Nile, dengue)	CSF and paired serum specimens	Detection of IgM antibodies in CSF and sera, virus isolation	Utility of PCR has not been comprehensively evaluated
4.	*Paramyxoviridae* (mumps, measles, Nipah)	CSF and paired serum specimens	Detection of IgM antibodies in CSF and sera, virus isolation	Utility of PCR has not been comprehensively evaluated

Picornaviruses - Diagnosis



- Enteroviruses
  - Laboratory
    - Clinical Chemistry
      - cerebrospinal fluid from CNS disease reveals
        - lymphocytic pleocytosis (25 - 500 cell/ml)
      - CSF glucose and protein
        - glucose normal or slightly depressed
        - protein normal or slightly elevated
    - Serology
      - detection of specific viral antibody in IgM fraction
      - four fold increase in IgG from acute to convelescence
  - Culture performed only for epidemiological confirmation
      - polioviruses from pharynx or feces
      - coxsackie or echoviruses from throat or feces
        - monkey kidney tissue culture
        - human embryo kidney tissue culture
      - culture virus is specifically identified with antibody assays



Enterovirus Lab Findings

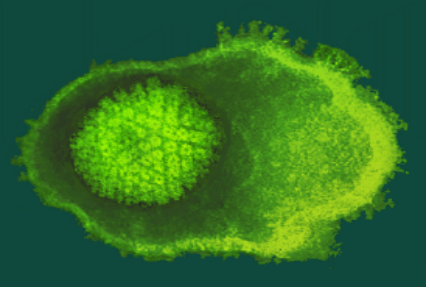
- CSF- findings typical of viral meningitis, with lymphocytic pleocytosis of generally <250 cells/mm3, with modest protein elevation generally <150 mg/dl, and normal glucose, viral cultures positive in 40-80% of cases but it usually takes 4-12 days to become positive, PCR is the most specific (close to 100%) and sensitive (97-100%) test and is positive in more than 2/3 of culture negative CSF in patients with aseptic meningitis
- Can also culture throat and stool specimens but this typically leads to a significant number of false positive results.
- Enterovirus IgM ELISA testing of serum may support a diagnosis of recent enteroviral infection.

Enteroviruses

- **Comparison of RT-PCR vs. Viral Culture**
  - **59 inpatient CSF samples tested**

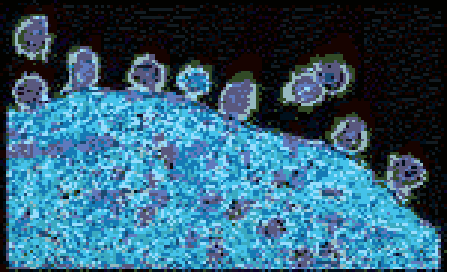
Result	RT-PCR	Culture
Pos	37	22
Neg	22	37

  - **Sensitivity of CSF viral culture = 60%**
  - **Culture time to detection = 3 – 5 days**
  - **RT-PCR time to detection = 3 – 4 hours**



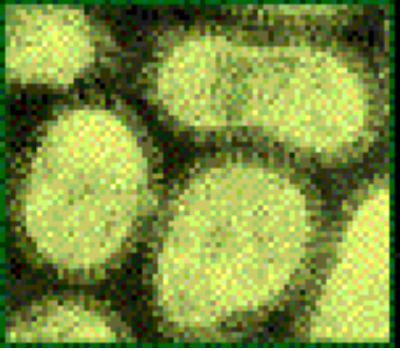
HSV Diagnosis

- CSF- findings typical of viral meningitis, with lymphocytic pleocytosis, modest elevation in protein, and normal glucose.
- Viral cultures are positive in approx. 80% of patients with primary HSV meningitis, but less frequently positive in patients with recurrent HSV meningitis.
- HSV PCR of the CSF is the single most useful test for the evaluation of a patient with suspected HSV meningitis.



HIV Meningitis Diagnosis

- CSF- might show a lymphocytic pleocytosis, elevated protein, and normal glucose.
- CSF cultures are often positive, but are not available in most centers.
- detection of HIV plasma viremia by nucleic acid techniques can be used for diagnosis.



Mumps Diagnosis

- CSF- similar to other viral causes, but like LCM it can induce a lymphocytic pleocytosis with cell counts >1000/mm3 or a decreased glucose <50mg/dl, can isolate the virus from the CSF
- Clinical correlation is very helpful, ex. If the patient has parotitis or orchitis.

CMV Infection of the CNS

Diagnosis of CMV-related CNS disease is based upon clinical presentation, neuroradiological studies, CSF chemistries, serological testing, and culture and PCR of CSF

- **Clinical presentations of CMV-related CNS disease can be nonspecific**
- **CSF viral culture can be insensitive**
- **Qualitative DNA PCR can detect both latent and replicating virus**

- **Rapid diagnosis of viral meningitis by PCR impacts clinical management:**
  - **Earlier hospital discharge**
  - **Fewer additional diagnostic tests**
  - **Decreased antibiotic usage**
  - **Decreased overall health care costs**