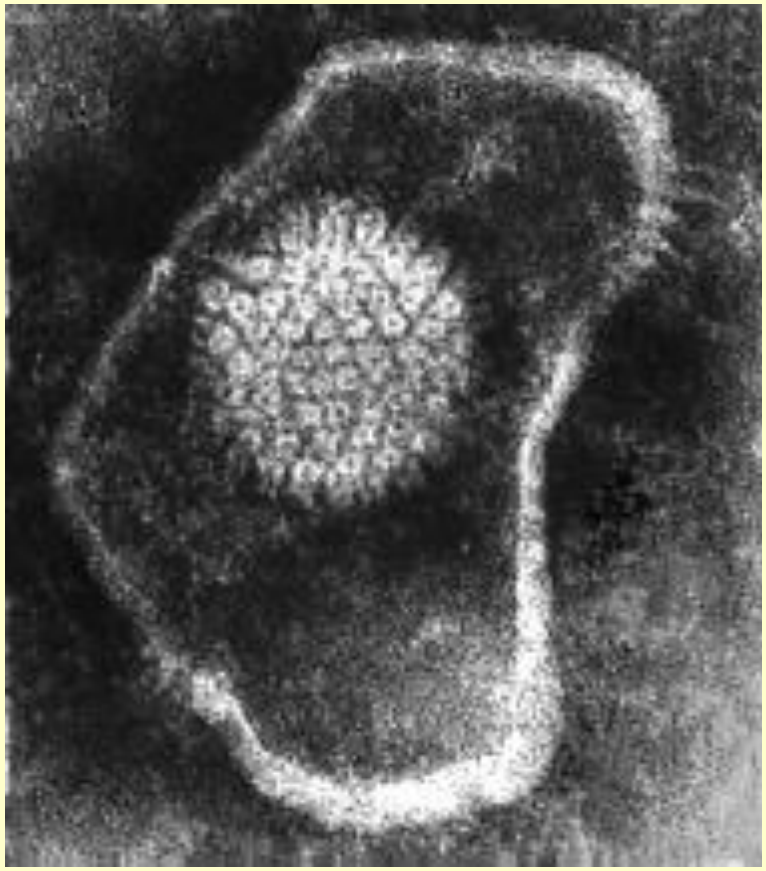
Herpesviruses

3rd class lab.no.16

Properties of herpesviruses

- Enveloped double stranded DNA viruses.
- Genome consists of long and short fragments which may be orientated in either direction, giving a total of 4 isomers.
- Three subfamilies:
  - Alphaherpesviruses - HSV-1, HSV-2, VZV
  - Betaherpesviruses - CMV, HHV-6, HHV-7
  - Gammaherpesviruses - EBV, HHV-8
- Set up latent or persistent infection following primary infection
- Reactivation are more likely to take place during periods of immunosuppression
- Both primary infection and reactivation are likely to be more serious in immunocompromised patients.

Herpesvirus Particle



(Linda Stannard, University of Cape Town, S.A.)

HSV-2 virus particle. Note that all herpesviruses have identical morphology and cannot be distinguished from each other under electron microscopy.

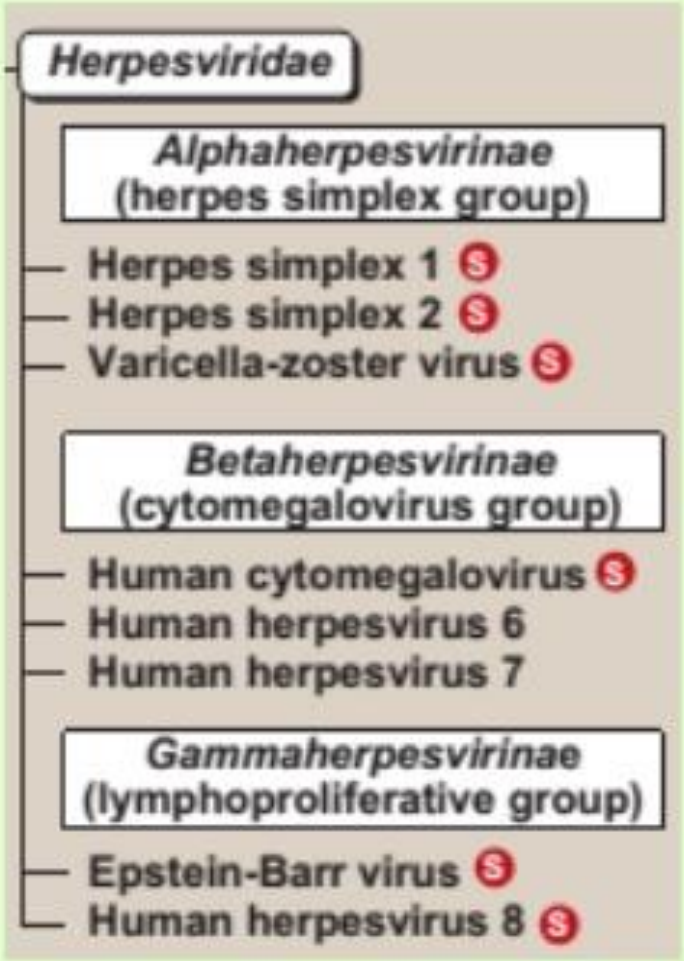
Human herpesviruses

They have common:

- Virion morphology
- Basic mode of replication
- Capacity to establish latent and recurrent infections, in case of EBV immortalizing infections
- Ubiquitous
- Usually cause benign disease especially in children
- In immunosuppressed people they cause significant morbidity and mortality

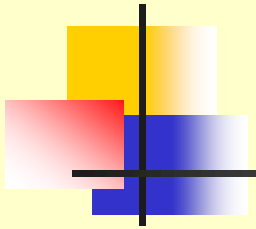
Classification

- Its also classified on the basis of on biologic characteristics:
  - **Alphaherpesvirinae (herpes simplex virus group)**
  - **Betaherpesvirinae (cytomegalovirus group)**
  - **Gammaherpesvirinae (lymphoproliferative group)**



Classification

- Eight human herpesvirus species are known.
  - **Herpes Simplex Virus type 1 (HSV-1)**
  - **Herpes Simplex Virus type 2 (HSV-2)**
  - **Varicella-Zoster Virus (VZV)**
  - **Cytomegalovirus (CMV)**
  - **Epstein-Barr Virus (EBV)**
  - **Human Herpes Virus type 6 (HHV-6)**
  - **Human Herpes Virus type 7 (HHV-7)**
  - **Human Herpes Virus type 8 (HHV-8)**



Herpes Simplex Viruses

Properties

- Belong to the alphaherpesvirus subfamily of herpesviruses
- Double stranded DNA enveloped virus with a genome of around 150 kb
- The genome of HSV-1 and HSV-2 share 50 - 70% homology.
- They also share several cross-reactive epitopes with each other. There is also antigenic cross-reaction with VZV.
- Man is the only natural host for HSV.

Epidemiology

- Generally HSV-1 causes infection above the belt and HSV-2 below the belt. In fact, 40% of clinical isolates from genital sores are HSV-1, and 5% of strains isolated from the facial area are HSV-2. This data is complicated by oral sexual practices.
- Following primary infection, 45% of orally infected individuals and 60% of patients with genital herpes will experience recurrences.
- The actual frequency of recurrences varies widely between individuals. The mean number of episodes per year is about 1.6.

Pathogenesis

- During the primary infection, HSV spreads locally and a short-lived viraemia occurs, whereby the virus is disseminated in the body. Spread to the to craniospinal ganglia occurs.
- The virus then establishes latency in the craniospinal ganglia.
- The exact mechanism of latency is not known, it may be true latency where there is no viral replication or viral persistence where there is a low level of viral replication.
- Reactivation - It is well known that many triggers can provoke a recurrence. These include physical or psychological stress, infection; especially pneumococcal and meningococcal, fever, irradiation; including sunlight, and menstruation.

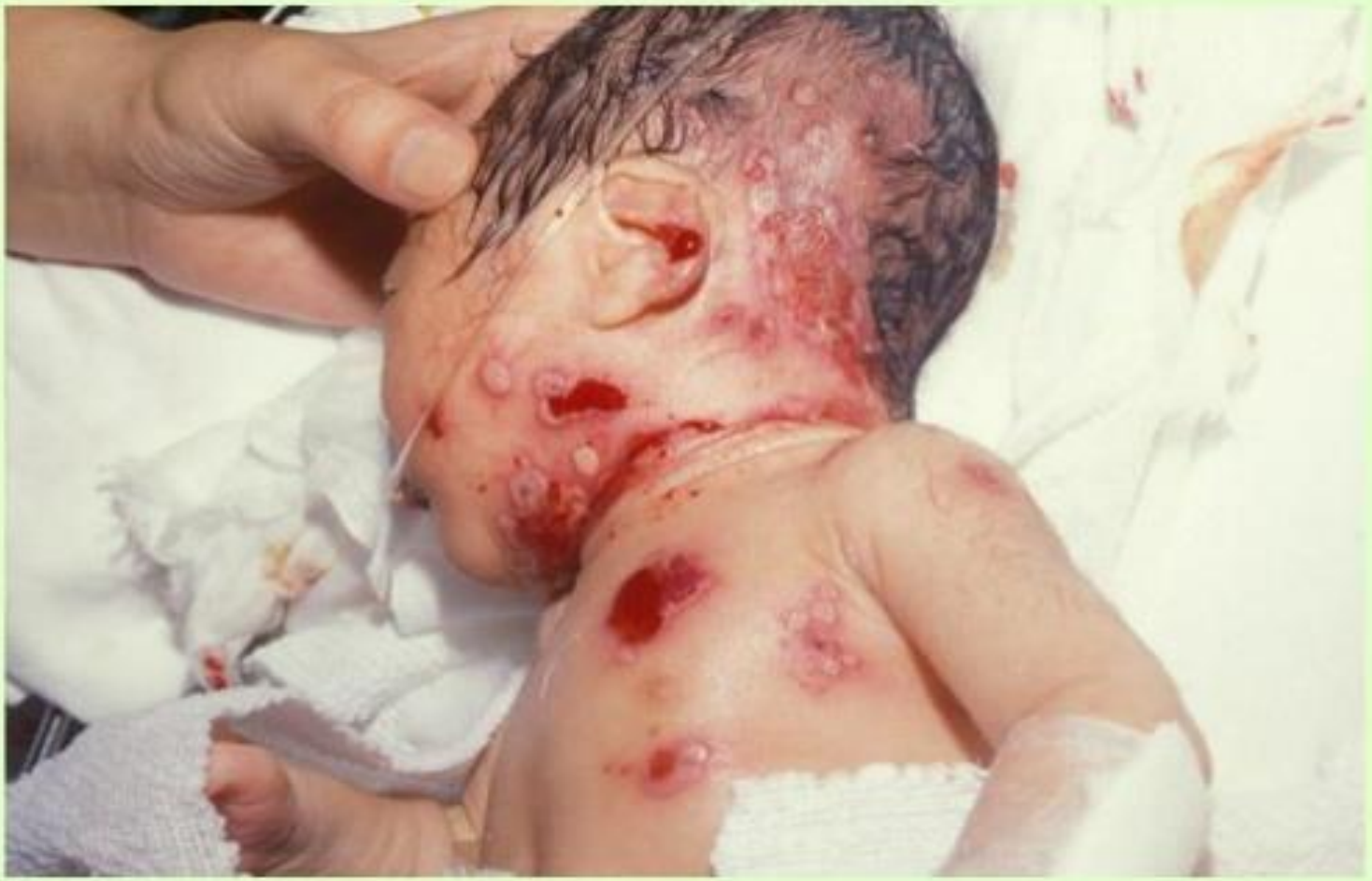
Clinical Manifestations

HSV is involved in a variety of clinical manifestations which includes ;-

1. Acute gingivostomatitis
2. Herpes Labialis (cold sore)
3. Ocular Herpes
4. Herpes Genitalis
5. Other forms of cutaneous herpes
7. Meningitis
8. Encephalitis
9. Neonatal herpes

Gingivostomatitis

Neonatal herpes



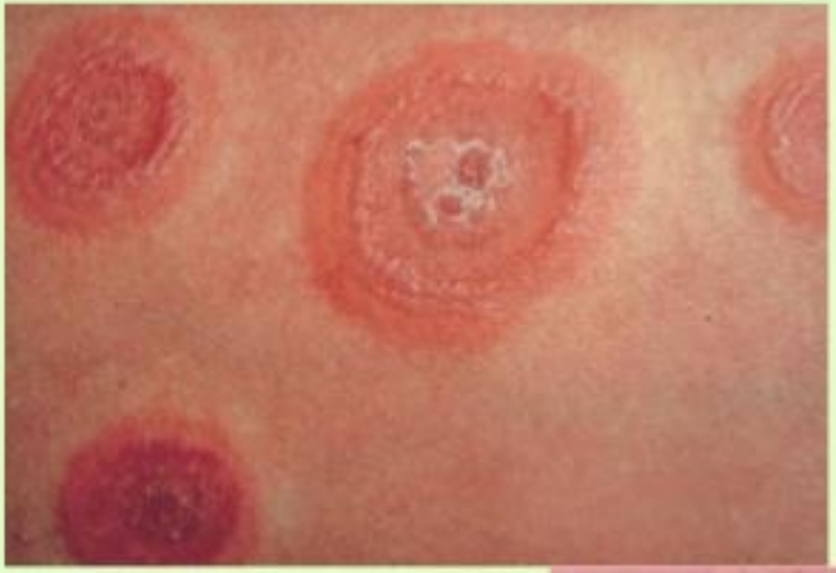
Other Manifestations

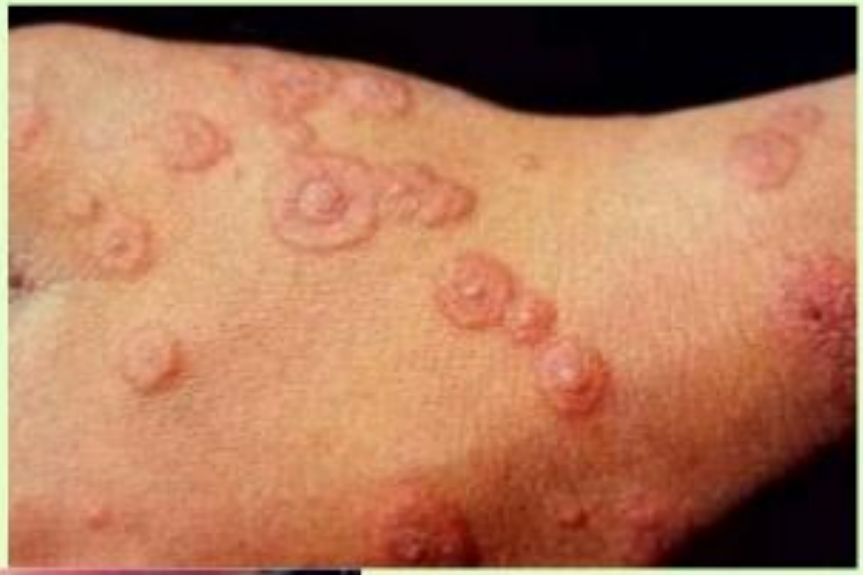
- Disseminated herpes simplex are much more likely to occur in immunocompromised individuals. The widespread vesicular resembles that of chickenpox. Many organs other than the skin may be involved e.g. liver, spleen, lungs, and CNS.
- Other cutaneous manifestations include
  - eczema herpeticum which is potentially a serious disease that occurs in patients with eczema.
  - Herpetic whitlow which arise from implantation of the virus into the skin and typically affect the fingers.
  - “zosteriform herpes simplex". This is a rare presentation of herpes simplex where HSV lesions appear in a dermatomal distribution similar to herpes zoster.

Erythema Multiforme

- Both HSV-1 and HSV-2 infections are associated with E.M.
- central red area surrounded by a ring of normal skin outside of which is a red ring
- "target" or "bull's eye" lesion.
- lesions are typically macular or papular
- occur symmetrically on the trunk, hands, and feet
- Many drugs, especially **sulfonamides** among the antimicrobial drugs, commonly cause erythema multiforme.
- Other associated MCO: *Mycoplasma pneumoniae* and viruses such as hepatitis B virus and hepatitis C virus.
- Erythema multiforme major, also known as **StevensJohnson syndrome**
  - Fever, erosive oral lesions,
  - Extensive desquamating skin lesions.

Erythema Multiforme





Laboratory Diagnosis

- Direct Detection
  - Electron microscopy of vesicle fluid - rapid result but cannot distinguish between HSV and VZV
  - Immunofluorescence of skin scrappings - can distinguish between HSV and VZV
  - PCR - now used routinely for the diagnosis of herpes simplex encephalitis and other herpes simplex infections.
- Virus Isolation
  - HSV-1 and HSV-2 are among the easiest viruses to cultivate. It usually takes only 1 - 5 days for a result to be available.
- Serology
  - Not that useful in the acute phase because it takes 1-2 weeks for before antibodies appear after infection. Used to document to recent infection.

Laboratory diagnosis

1. Smears

- Scrapings from base of Vesicle
- CSF,
- Saliva.

2. Serum

-Primary infection.

-ELISA most useful.

-CFT.

Microscopy:

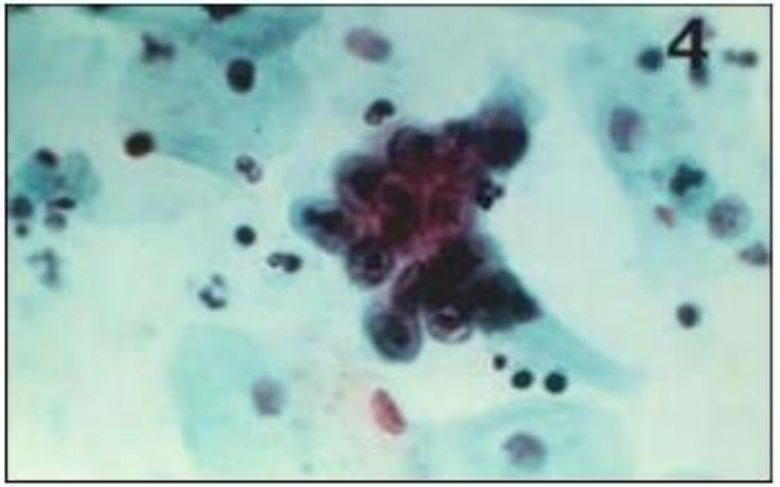
Tzanck smear

1 % Aqu. sol. of Toludine blue.

Multinucleated giant cells with faceted nuclei , ground glass chromatin.

(Tzanck cells)

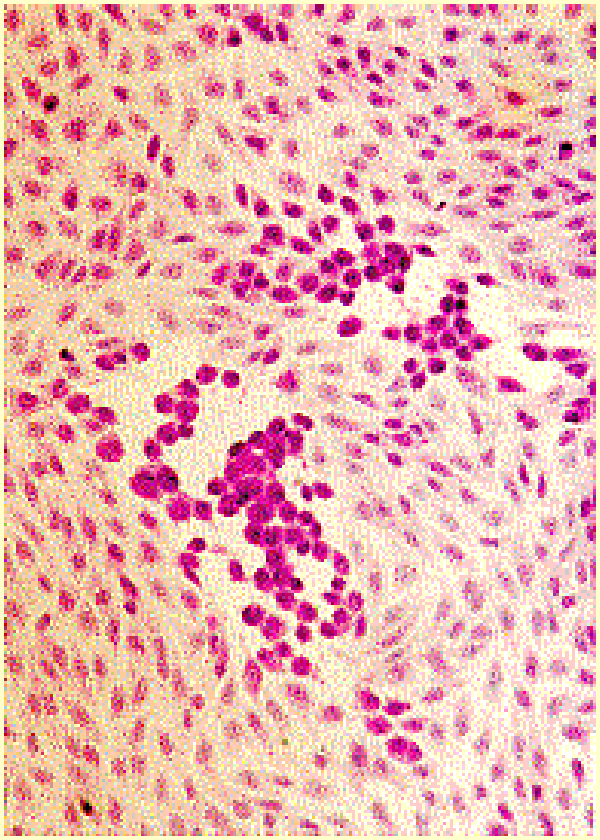
Best : **Giemsa, Papanicolou stain**



Laboratory Diagnosis

Virus Isolation

- **Viral culture (gold standard)**
  - **Preferred test if genital ulcers or other mucocutaneous lesions are present**
  - **Highly specific (>99%)**
  - **Sensitivity depends on stage of lesion; declines rapidly as lesions begin to heal**
  - **Positive more often in primary infection (80%–90%) than with recurrences (30%)**



Cytopathic Effect of HSV in cell culture: Note the ballooning of cells. (Linda Stannard, University of Cape Town, S.A.)

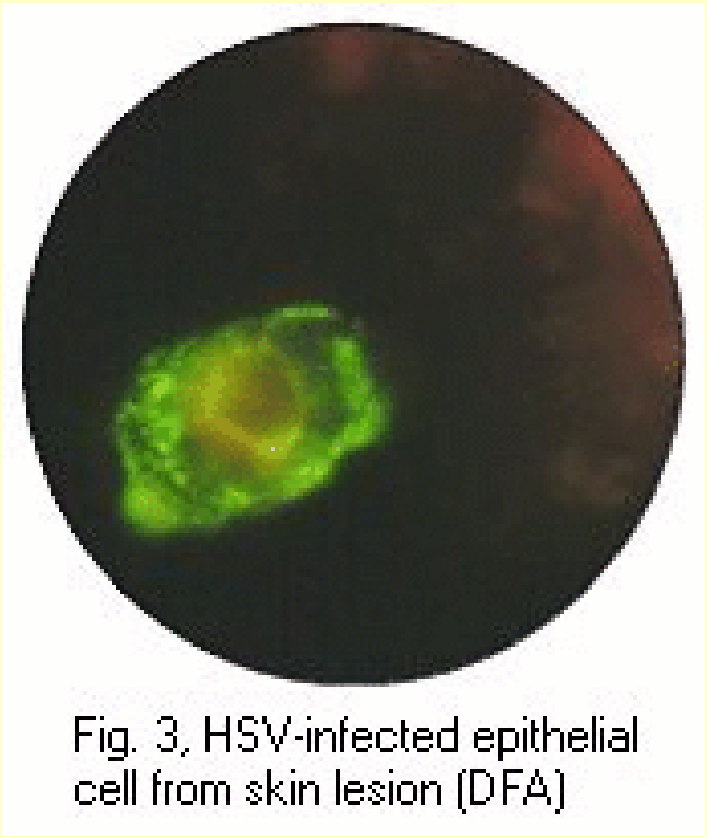
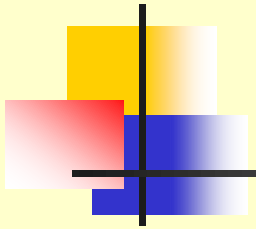


Fig. 3, HSV-infected epithelial cell from skin lesion (DFA)

Positive immunofluorescence test for HSV antigen in epithelial cell. (Virology Laboratory, New-Yale Haven Hospital)



Varicella- Zoster Virus

Properties

- Belong to the Alphaherpesviruses subfamily of herpesviruses
- Double stranded DNA enveloped virus
- Genome size 125 kbp, long and short fragments with a total of 4 isometric forms.
- One antigenic serotype only, although there is some cross reaction with HSV.

Epidemiology

- Primary varicella is an endemic disease. Varicella is one of the classic diseases of childhood, with the highest prevalence occurring in the 4 - 10 years old age group.
- Varicella is highly communicable, with an attack rate of 90% in close contacts.
- Most people become infected before adulthood but 10% of young adults remain susceptible.
- Herpes zoster, in contrast, occurs sporadically and evenly throughout the year.

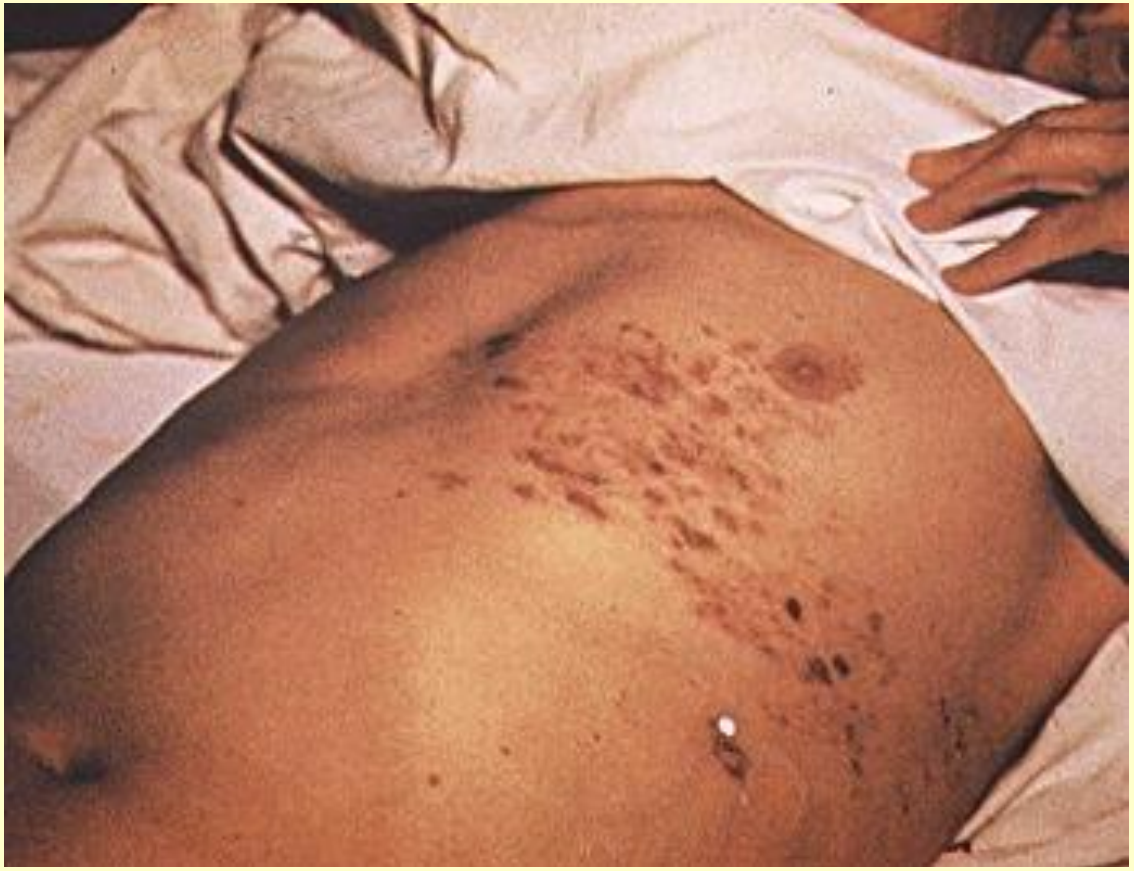
epidemiology

- Primary infection results in varicella (chickenpox)
- Incubation period of 14-21 days
- Presents fever, lymphadadenopathy. a widespread vesicular rash.
- The features are so characteristic that a diagnosis can usually be made on clinical grounds alone.
- Complications are rare but occurs more frequently and with greater severity in adults and immunocompromised patients.
- Most common complication is secondary bacterial infection of the vesicles.
- Severe complications which may be life threatening include viral pneumonia, encephalititis, and haemorrhagic chickenpox.

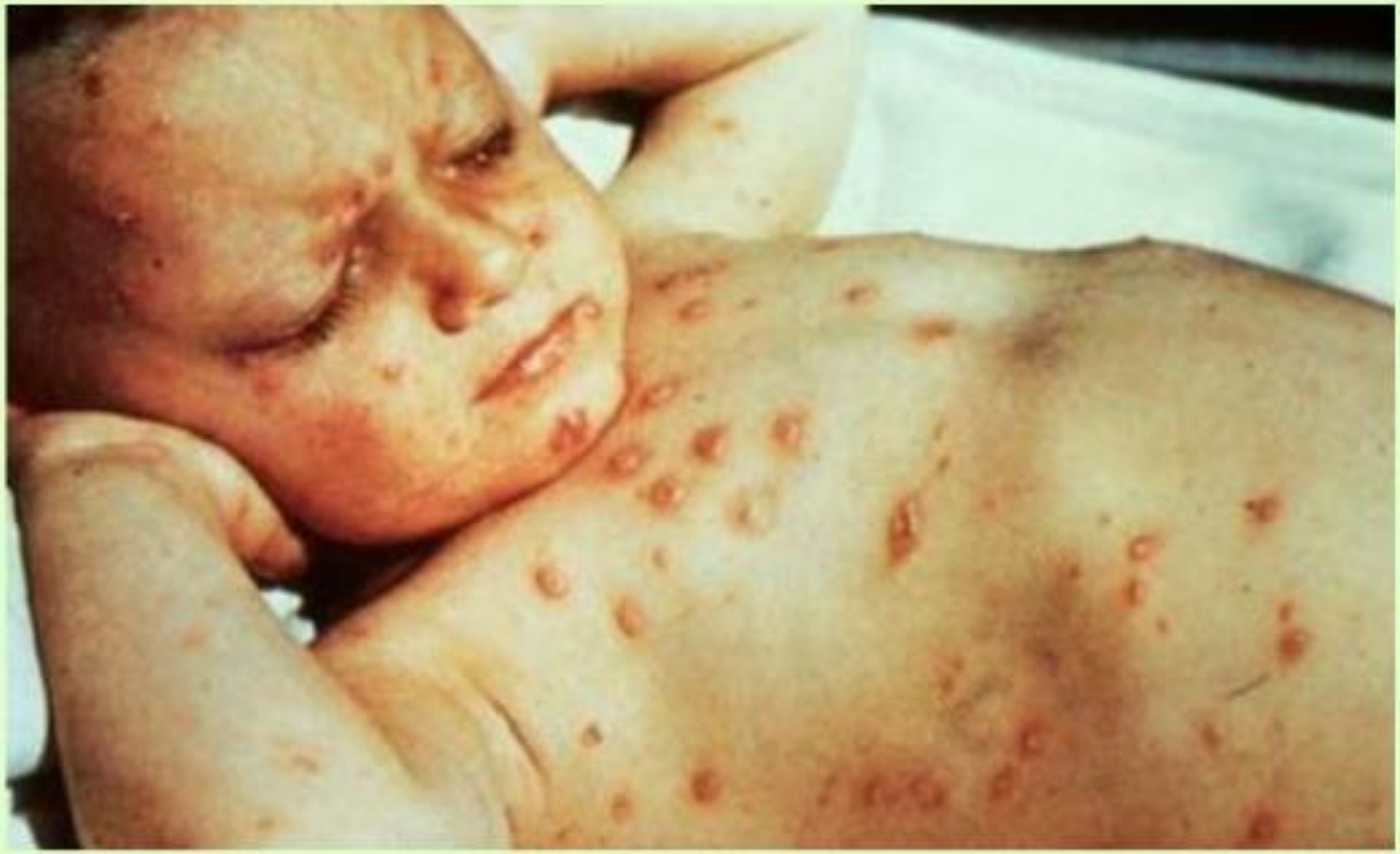
Herpes Zoster (Shingles)

- Herpes Zoster mainly affect a single dermatome of the skin.
- It may occur at any age but the vast majority of patients are more than 50 years of age.
- The latent virus reactivates in a sensory ganglion and tracks down the sensory nerve to the appropriate segment.
- There is a characteristic eruption of vesicles in the dermatome which is often accompanied by intensive pain which may last for months (postherpetic neuralgia)
- Herpes zoster affecting the eye and face may pose great problems.
- As with varicella, herpes zoster in a far greater problem in immunocompromised patients in whom the reactivation occurs earlier in life and multiple attacks occur as well as complications.
- Complications are rare and include encephalitis and disseminated herpes zoster.

Shingles



VZV - Chicken Pox



Congenital VZV Infection

- 90% of pregnant women already immune, therefore primary infection is rare during pregnancy.
- Primary infection during pregnancy carries a greater risk of severe disease, in particular pneumonia.

First 20 weeks of Pregnancy

- Up to 3% chance of transmission to the fetus, recognised congenital varicella syndrome;
  - Scarring of skin
  - Hypoplasia of limbs
  - CNS and eye defects
  - Death in infancy normal

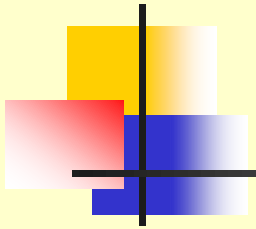
Laboratory Diagnosis

The clinical presentations of varicella or zoster are so characteristic that laboratory confirmation is rarely required. Laboratory diagnosis is required only for atypical presentations, particularly in the immunocompromised.

- Virus Isolation - rarely carried out as it requires 2-3 weeks for a results.
- Direct detection - electron microscopy may be used for vesicle fluids but cannot distinguish between HSV and VZV. Immunofluorescense on skin scrappings can distinguish between the two. PCR assays for VZV are available and have been reported to be particularly useful in the diagnosis of VZV meningoencephalitis from CSF specimens.
- Serology - the presence of VZV IgG is indicative of past infection and immunity. The presence of IgM is indicative of recent primary infection.

Cytopathic Effect of VZV

Cytopathic Effect of VZV in cell culture: Note the ballooning of cells. (Coutesy of Linda Stannard, University of Cape Town, S.A.)



Cytomegalovirus

Properties

- Belong to the betaherpesvirus subfamily of herpesviruses
- double stranded DNA enveloped virus
- Nucleocapsid 105nm in diameter, 162 capsomers
- The structure of the genome of CMV is similar to other herpesviruses, consisting of long and short segments which may be orientated in either direction, giving a total of 4 isomers.
- A large no. of proteins are encoded for, the precise number is unknown.

Epidemiology

- CMV is one of the most successful human pathogens, it can be transmitted vertically or horizontally usually with little effect on the host.
- Transmission may occur in utero, perinatally or postnatally. Once infected, the person carries the virus for life which may be activated from time to time, during which infectious virions appear in the urine and the saliva.
- Reactivation can also lead to vertical transmission. It is also possible for people who have experienced primary infection to be reinfected with another or the same strain of CMV, this reinfection does not differ clinically from reactivation.
- In developed countries with a high standard of hygiene, 40% of adolescents are infected and ultimately 70% of the population is infected. In developing countries, over 90% of people are ultimately infected.

Pathogenesis

- Once infected, the virus remains in the person for life and my be reactivated from time to time, especially in immunocompromised individuals.
- The virus may be transmitted in utero, perinatally, or postnatally. Perinatal transmission occurs.
- Perinatal infection is acquired mainly through infected genital secretions, or breast milk. Overall, 2 - 10% of infants are infected by the age of 6 months worldwide. Perinatal infection is thought to be 10 times more common than congenital infection.
- Postnatal infection mainly occurs through saliva. Sexual transmission may occur as well as through blood and blood products and transplanted organ.

Clinical Manifestations

- Congenital infection - may result in cytomegalic inclusion disease
- Perinatal infection - usually asymptomatic
- Postnatal infection - usually asymptomatic. However, in a minority of cases, the syndrome of infectious mononucleosis may develop which consists of fever, lymphadenopathy, and splenomegaly. The heterophil antibody test is negative although atypical lymphocytes may be found in the blood.
- Immunocompromised patients such as transplant recipients and AIDS patients are prone to severe CMV disease such as pneumonitis, retinitis, colitis, and encephalopathy.
- Reactivation or reinfection with CMV is usually asymptomatic except in immunocompromised patients.

Cytomegalic Inclusion Disease

- CNS abnormalities - microcephaly, mental retardation, spasticity, epilepsy, periventricular calcification.
- Eye - choroidoretinitis and optic atrophy
- Ear - sensorineural deafness
- Liver - hepatosplenomegaly and jaundice which is due to hepatitis.
- Lung - pneumonitis
- Heart - myocarditis
- Thrombocytopenic purpura, Haemolytic anaemia
- Late sequelae in individuals asymptomatic at birth - hearing defects and reduced intelligence.

Laboratory Diagnosis (1)

- Direct detection
  - biopsy specimens may be examined histologically for CMV inclusion antibodies or for the presence of CMV antigens. However, the sensitivity may be low.
  - The pp65 CMV antigenaemia test is now routinely used for the rapid diagnosis of CMV infection in immunocompromised patients.
  - PCR Assays, including real-time quantitative PCR are now used in many centers but the lack of standardization of PCR protocols hindered the comparison of data between centers.

CMV pp65 antigenaemia test

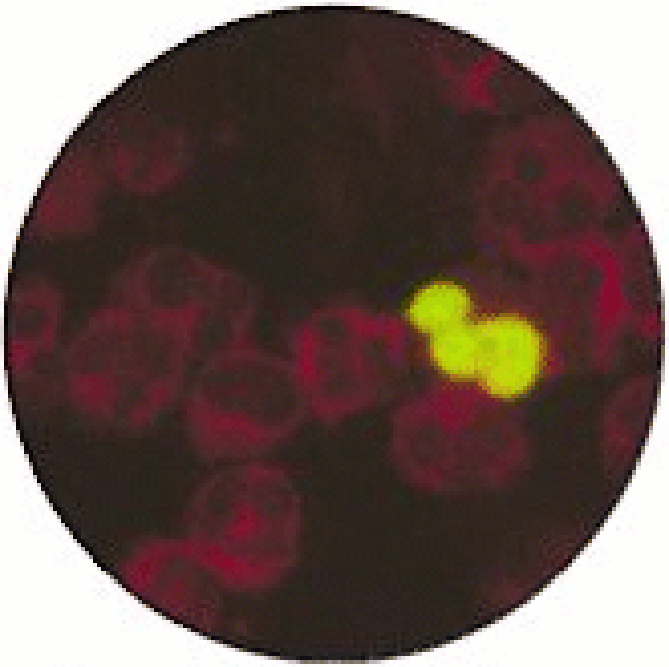


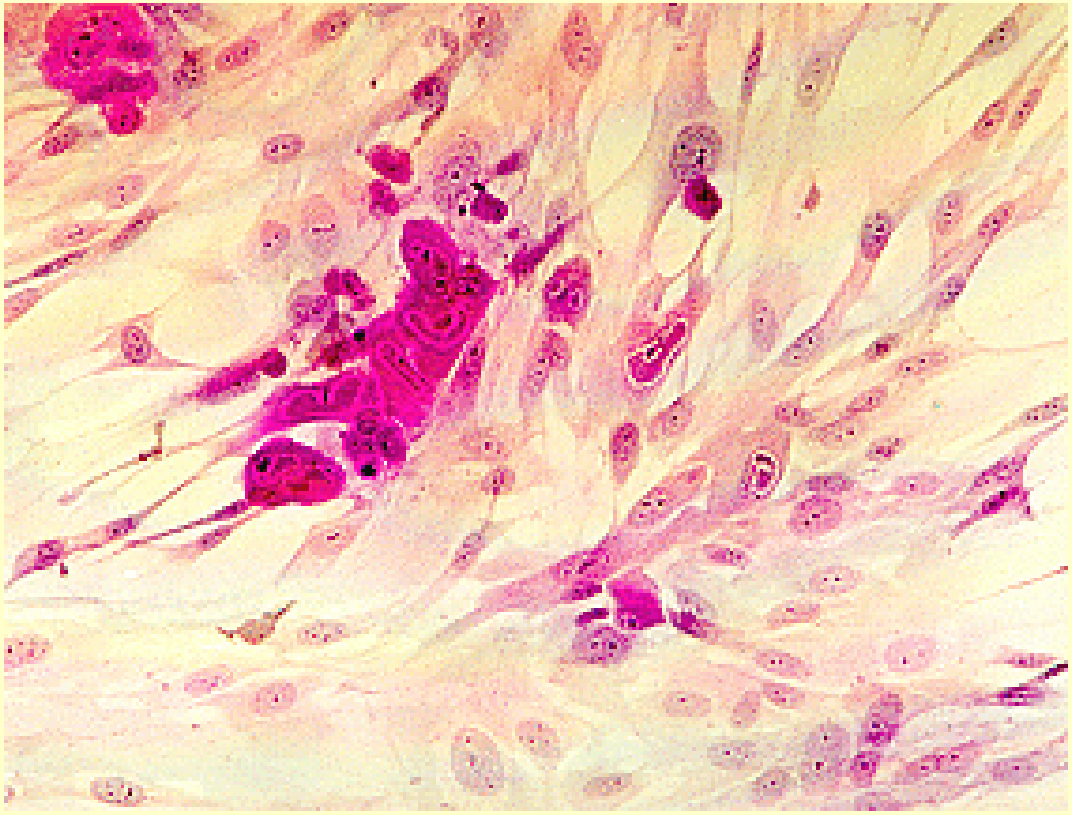
Figure 4 CMV pp65 antigens detected in nuclei of peripheral blood neutrophils

(Virology Laboratory, New-Yale Haven Hospital)

Laboratory Diagnosis (2)

- Virus Isolation
  - conventional cell culture is regarded as gold standard but requires up to 4 weeks for result.
  - More useful are rapid culture methods such as the DEAFF test which can provide a result in 24-48 hours.
- Serology
  - the presence of CMV IgG antibody indicates past infection.
  - The detection of IgM is indicative of primary infection although it may also be found in immunocompromised patients with reactivation.

Cytopathic Effect of CMV



(Courtesy of Linda Stannard, University of Cape Town, S.A.)

DEAFF test for CMV

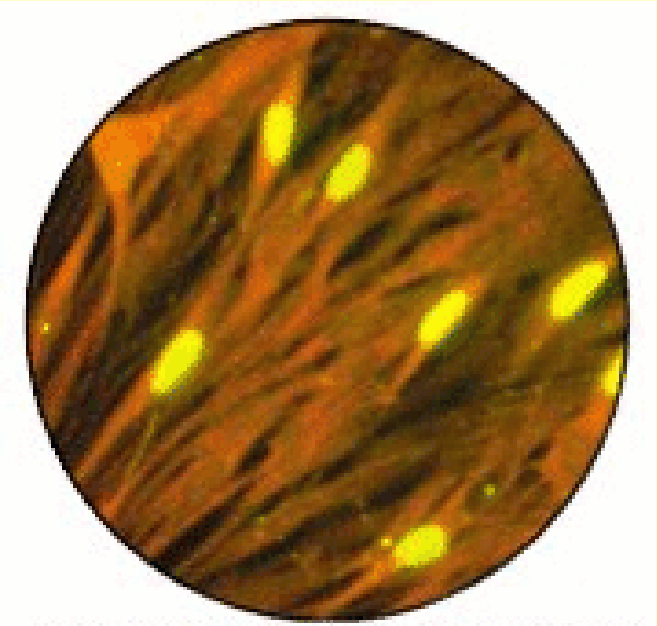


Fig. 2, CMV centrifugation culture fixed and stained 16 hrs after inoculation showing viral proteins in nuclei of infected human fibroblast cells

(Virology Laboratory, New-Yale Haven Hospital)

Specimens for Laboratory Diagnosis

	Site for virus culture				Serology	
	Urine	Saliva	Blood	Tissue affected	IgG	IgM
Neonates	+	+	-	-	-	+
Adults	+	-	+	-	+	+
Pregnant women	-	-	-	-	+	+
Immunocompromised	+	+	+	+	+	-

Epstein-Barr Virus

Epstein-Barr Virus (EBV)

- Belong to the gammaherpesvirus subfamily of herpesviruses
- Nucleocapsid 100 nm in diameter, with 162 capsomers
- Membrane is derived by budding of immature particles through cell membrane and is required for infectivity.
- Genome is a linear double stranded DNA molecule with 172 kbp
- The viral genome does not normally integrate into the cellular DNA but forms circular episomes which reside in the nucleus.
- The genome is large enough to code for 100 - 200 proteins but only a few have been identified.

Epidemiology

- Two epidemiological patterns are seen with EBV.
- In developed countries, 2 peaks of infection are seen : the first in very young preschool children aged 1 - 6 and the second in adolescents and young adults aged 14 - 20 Eventually 80-90% of adults are infected.
- In developing countries, infection occurs at a much earlier age so that by the age of two, 90% of children are seropositive.
- The virus is transmitted by contact with saliva, in particularly through kissing.

Pathogenesis

- Once infected, a lifelong carrier state develops whereby a low grade infection is kept in check by the immune defenses.
- Low grade virus replication and shedding can be demonstrated in the epithelial cells of the pharynx of all seropositive individuals.
- EBV is able to immortalize B-lymphocytes in vitro and in vivo
- Furthermore a few EBV-immortalized B-cells can be demonstrated in the circulation which are continually cleared by immune surveillance mechanisms.
- EBV is associated with several very different diseases where it may act directly or one of several co-factors.

Disease Association

1. Infectious Mononucleosis
2. Burkitt's lymphoma
3. Nasopharyngeal carcinoma
4. Lymphoproliferative disease and lymphoma in the immunosuppressed.
5. X-linked lymphoproliferative syndrome
6. Chronic infectious mononucleosis
7. Oral leukoplakia in AIDS patients
8. Chronic interstitial pneumonitis in AIDS patients.

Infectious Mononuclosis

- Primary EBV infection is usually subclinical in childhood. However in adolescents and adults, there is a 50% chance that the syndrome of infectious mononucleosis (IM) will develop.
- IM is usually a self-limited disease which consists of fever, lymphadenopathy and splenomegaly. In some patients jaundice may be seen which is due to hepatitis. Atypical lymphocytes are present in the blood.
- Complications occur rarely but may be serious e.g. splenic rupture, meningoencephalitis, and pharyngeal obstruction.
- In some patients, chronic IM may occur where eventually the patient dies of lymphoproliferative disease or lymphoma.
- Diagnosis of IM is usually made by the heterophil antibody test and/or detection of EBV IgM.
- There is no specific treatment.

Nasopharyngeal Carcinoma

- Nasopharyngeal carcinoma (NPC) is a malignant tumour of the squamous epithelium of the nasopharynx. It is very prevalent in S. China, where it is the commonest tumour in men and the second commonest in women.
- The tumour is rare in most parts of the world, though pockets occur in N. and C. Africa, Malaysia, Alaska, and Iceland.
- Multiple copies of EBV genome and EBV EBNA-1 antigen can be found in cells of undifferentiated NPC. Patients with NPC have high titres of antibodies against various EBV antigens.
- Besides EBV there appears to be a number of environmental and genetic cofactors in NPC.
- NPC usually presents late and thus the prognosis is poor.
- In theory NPC can be prevented by vaccination.

Immunocompromised Patients

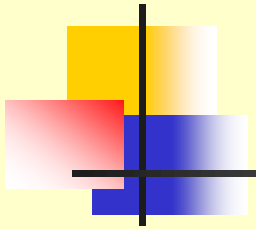
- After primary infection, EBV maintains a steady low grade latent infection in the body. Should the person become immunocompromised, the virus will reactivate. In a few cases, lymphoproliferative lesions and lymphoma may develop. These lesions tend to be extranodal and in unusual sites such as the GI tract or the CNS.
- Transplant recipients e.g. renal - EBV is associated with the development of lymphoproliferative disease and lymphoma.
- AIDS patients - EBV is associated with oral leukoplakia and with various Non-Hodgekin's lymphoma.
- Ducan X-linked lymphoproliferative syndrome - this condition occurs exclusively in males who had inherited a defective gene in the X-chromosome . This condition accounts for half of the fatal cases of IM.

Diagnosis

- Acute EBV infection is usually made by the heterophil antibody test and/or detection of anti-EBV VCA IgM.
- Cases of Burkitt's lymphoma should be diagnosed by histology. The tumour can be stained with antibodies to lambda light chains which should reveal a monoclonal tumour of B-cell origin. In over 90% of cases, the cells express IgM at the cell surface.
- Cases of NPC should be diagnosed by histology.
- The determination of the titre of anti-EBV VCA IgA in screening for early lesions of NPC and also for monitoring treatment.
- A patient with with non-specific ENT symptoms who have elevated titres of EBV IgA should be given a thorough examination.

EBV - Diagnosis

- Clinical picture
- Complete Blood Cell Count – Atypical lymphocytes
- **Serology**
  - Expensive
  - Demonstrate antibody to viral capsid antigen (VCA) which rises quickly and persists for life.
  - Antibodies to EBNAs rise later and decrease in about 1 month



Other Human Herpes Viruses

Properties of HHV-6 and 7

- Belong to the betaherpesvirus subfamily of herpesviruses
- Double stranded DNA genome of 170 kbp
- The main target cell is the T-lymphocyte, although B-lymphocytes may also be infected.
- HHV-6 and HHV-7 share limited nucleotide homology and antigenic cross-reactivity.
- It is thought that HHV-6 and HHV-7 are related to each other in a similar manner to HSV-1 and HSV-2.

Epidemiology and Pathogenesis

- HHV-6 and HHV-7 are ubiquitous and are found worldwide.
- They are transmitted mainly through contact with saliva and through breast feeding.
- HHV-6 and HHV-7 infection are acquired rapidly after the age of 4 months when the effect of maternal antibody wears off.
- By the time of adulthood, 90-99% of the population had been infected by both viruses.
- Like other herpesviruses, HHV-6 and HHV-7 remains latent in the body after primary infection and reactivates from time to time.

Clinical Manifestations (1)

- Primary HHV-6 infection is associated with Roseala Infantum, which is a classical disease of childhood.
- Most cases occur in infants between the ages of 4 months and two years.
- A spiking fever develops over a period of 2 days followed by a mild rash. The fever is high enough to cause febrile convulsions.
- There are reports that the disease may be complicated by encephalitis.

Clinical Manifestations (2)

- If primary infection is delayed until adulthood, there is a small chance that an infectious mononucleosis-like disease may develop in a similar manner to EBV and CMV.
- There is no firm evidence linking HHV-6 to lymphomas or lymphoproliferative diseases.
- There is no firm disease association with HHV-7 at present.
- Although both viruses may be reactivated in immunocompromised patients, it is yet uncertain whether they cause significant disease since CMV is almost invariably present.

Diagnosis and Management

- Rosela Infantum has a very characteristic presentation and a diagnosis can usually be made on clinical grounds alone.
- Therefore very few virology laboratories offer a diagnostic service for HHV-6 or HHV-7 infection.
- The technique for virus isolation is complicated and thus not practicable as a routine diagnostic procedure.
- Therefore serology is the mainstay of diagnosis where specific IgM and IgG are detected.
- There is no specific antiviral treatment for HHV-6 infection.

Human Herpes Virus 8

- Belong to the gammaherpesviruses subfamily of herpesviruses
- Originally isolated from cells of Kaposi's sarcoma (KS)
- Now appears to be firmly associated with Kaposi's sarcoma as well as some lesser known malignancies such as Castleman's disease and primary effusion lymphomas
- HHV-8 DNA is found in almost 100% of cases of Kaposi's sarcoma
- Most patients with KS have antibodies against HHV-8
- The seroprevalence of HHV-8 is low among the general population but is high in groups of individuals susceptible to KS, such as homosexuals.
- Unlike other herpesviruses, HHV-8 does not have a ubiquitous distribution.

Kaposi's Sarcoma