Virology Review 2020

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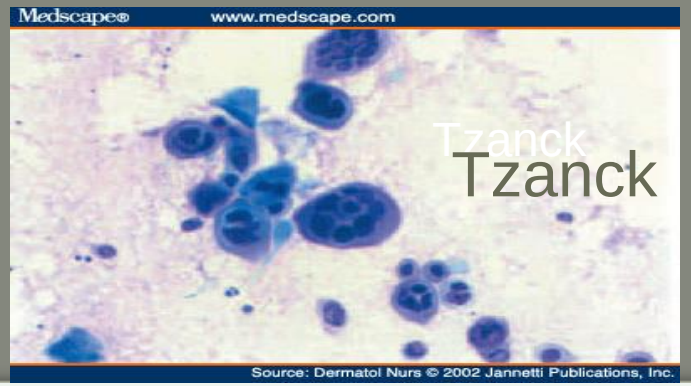
Diagnostic Techniques (old and new) in the Virology Laboratory

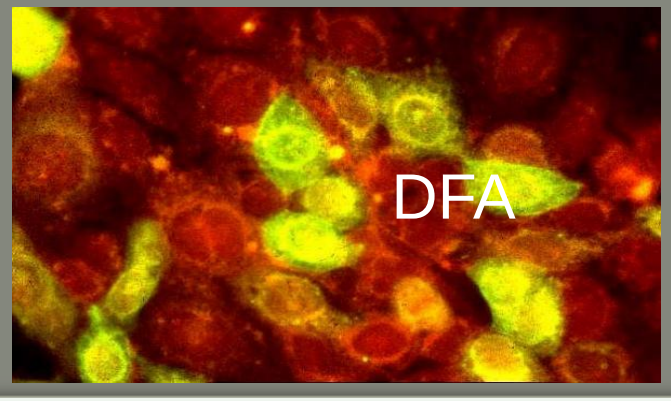
1. Direct Staining Specimen for Antigen
2. Enzyme Immunoassay
3. Viral Cell Culture
4. Molecular Amplification

Stains to detect virus antigen

- Direct Fluorescent antibody (DFA) stain
  - Collect cells onto slide from base of fresh vesicular lesion
  - Stain with antibody specific for HSV 1/2 and/or VZV
    - Look for fluorescent cells (virus infected) using fluorescence microscope
  - Sensitivity @ 80% for HSV 1, HSV 2, and VZV detection

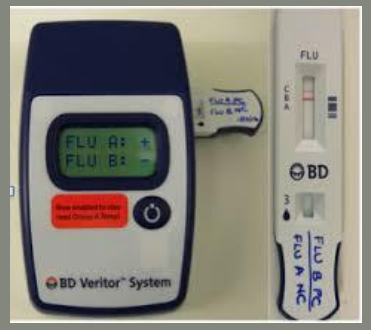
- Tzanck prep
  - Giemsa stained cells from lesion /examine for multinucleated giant cells consistent with Herpes virus family
  - Sensitivity @ 50% / Cannot differentiate HSV 1, HSV 2, or VZV



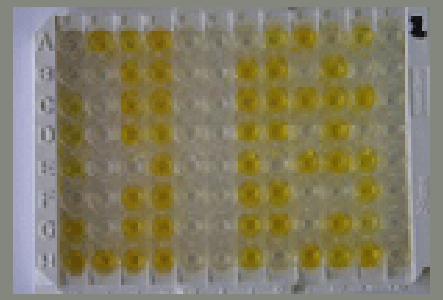


Detection of Viral Antigens using EIA

- Enzyme immunoassay (EIA) –
  - Antigen in specimen forms a complex with test antibody, then a color producing substrate binds to Ag/Aby complex
  - Rapid point of care test (20 min) , moderate sensitivity (70%), specificity adequate during high prevalence viral season
  - Used most often for:
    - Detection of non culturable viruses such as Rotavirus in stool specimens
    - Influenza A /B, Respiratory syncytial virus (RSV), SARS-CoV-2
- Membrane lateral flow EIA



Liquid/in-well EIA

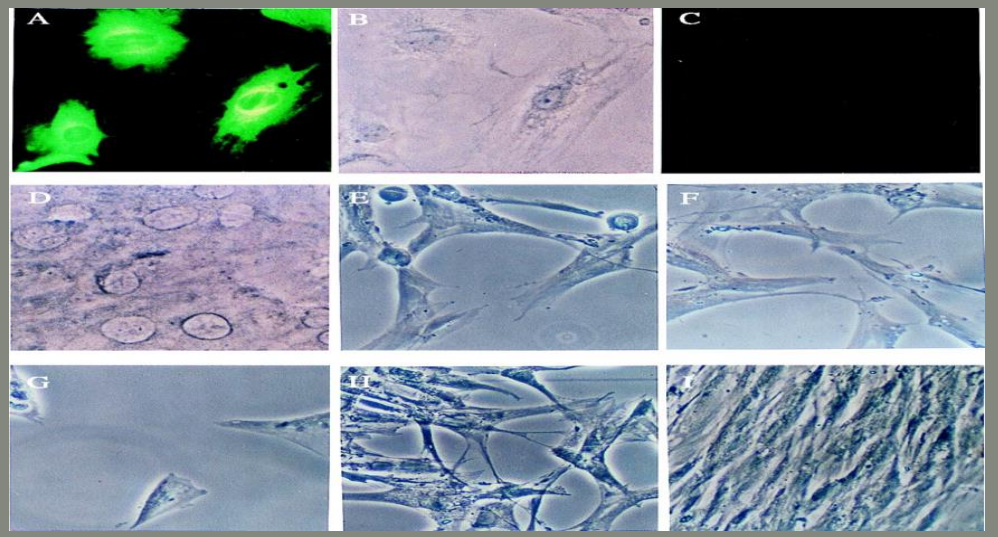


Viral Cell Culture

- Inner wall of tube/vessel coated with monolayer of cells grown in enriched liquid growth media
- Three types of cell lines:
  - **Primary** – obtained directly from animal or human organ and placed into culture vial , will only survive one subculture
    - Example: Rhesus monkey kidney-RMK
  - **Diploid – semi continuous** cell lines– Can survive 20 – 50 subcultures
    - Example: Human diploid fibroblast cells (MRC-5-Microbiology Research Council 5)
  - **Continuous** cell lines – survive continuous passage
    - Example: Tumor lineages such as HEp-2 and HeLa

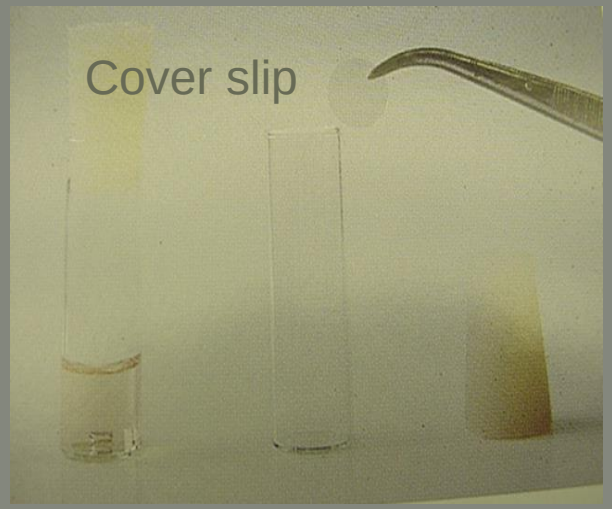
Viral Cell Culture

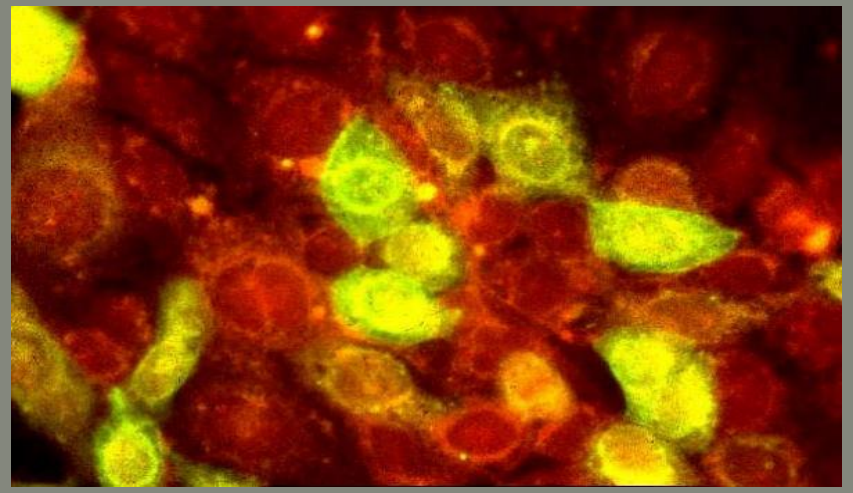
- Patient specimens inoculated onto cell monolayers, incubated for specified number of days, then read under light microscopy for "**Cytopathic effect**" – the effect viral growth has on the cell line
  - The pattern of destruction of the cell monolayer is specific for each virus type and the time in which destruction occurs is virus specific.



Spin Down Shell Vial Culture

- Technique created to speed up viral cell culture
- Cell monolayer is created on a coverslip
- Specimen inoculated into vial with coverslip and media
- Centrifuge vial to rapidly induce virus invasion of cells
- Incubate @ 35*C for 24-72 hours
- Direct fluorescent antibody staining using early virus antigens





Molecular Amplification

- Molecular Amplification of DNA or RNA (Qualitative)
  - Rapid/Sensitive/Specific detection method for numerous viruses
  - Direct amplification of DNA
  - RNA viruses - RT-PCR – reverse transcriptase enzyme transcribes complimentary copies of DNA using the RNA template
  - More rapid and exceeds sensitivity of viral culture. Has become the Standard of Practice for detection of many viruses:
    - Respiratory viruses
    - Encephalitis viruses
    - Lesion viruses
  - Tests of diagnosis not cure – can continue to shed residual virus for 7 – 30 days after initial positive test

Molecular quantitative assays

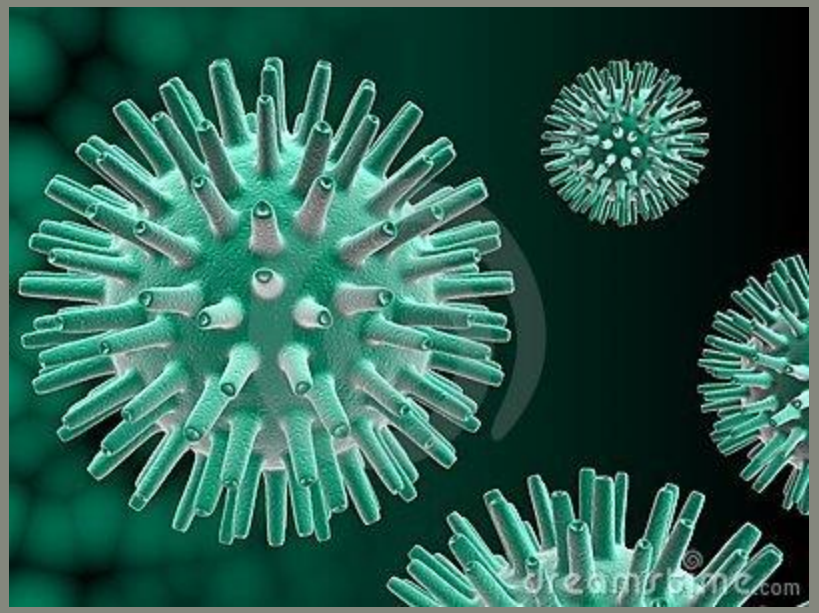
- CMV, Hepatitis B and C and HIV

Specimen collection and Transport

- Viral transport media (VTM) or Universal transport media (UTM) - Hanks balanced salt solution with antibiotics to prevent bacterial overgrowth
  - Transport of swab collected specimens: lesions, mucous membranes, nasopharyngeal & throat

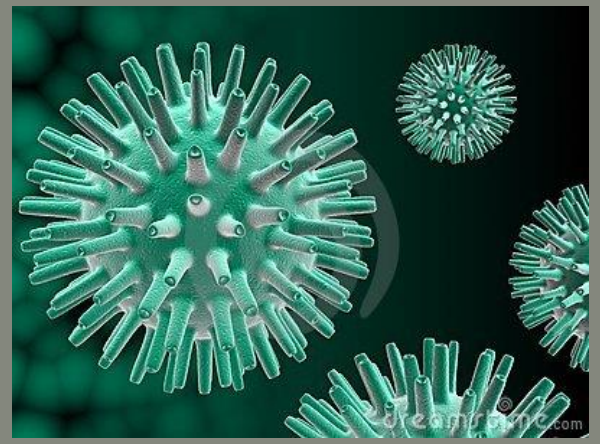
- **Short term** transport media storage 4˚C
- **Long term** transport(>72hours) media storage at -70˚C
- VTM/UTM filtered (45nm filter) to eliminate bacteria in specimen prior to being placed onto cell monolayer

Herpesviridae



Herpes Viruses /Herpesviridae

- Double stranded DNA virus
- Eight human Herpes viruses
  - Herpes simplex 1
  - Herpes simplex 2
  - Varicella Zoster
  - Epstein Barr
  - Cytomegalovirus
  - Human Herpes 6, 7, and 8



- Latency (hallmark of Herpes viruses) occurs within small numbers of specific kinds of cells, the cell type is different for each Herpes virus

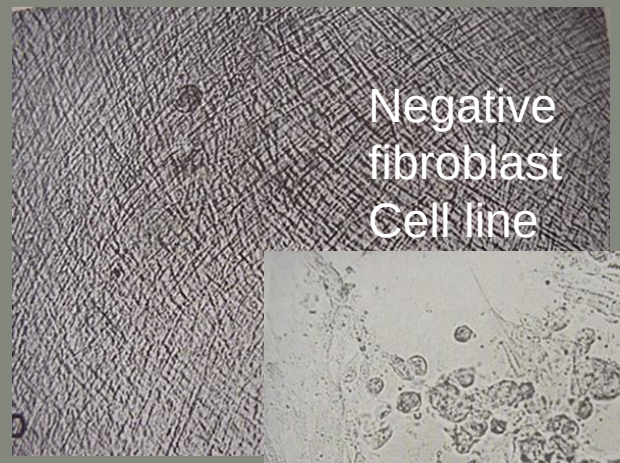
Herpes simplex virus 1 and 2

- Transmission: direct contact/secretions
- Latency: dorsal root ganglia
- Infection sites:
  - Gingivostomatitis - common primary infection
  - Herpes labialis
  - Ocular
  - Encephalitis
  - Neonatal – viral surface cultures should be performed for surveillance and diagnosis
  - Disseminated disease in immune suppressed

Therapy – Acyclovir, Valacyclovir

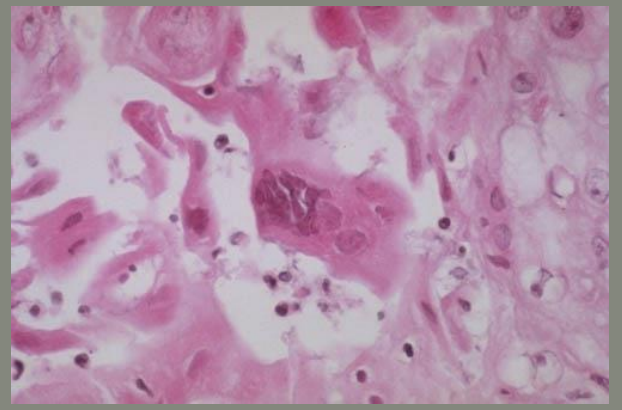
Herpes Simplex diagnosis

- Viral Cell Culture
  - Produce CPE within 24-48 hr
  - Human diploid fibroblast (MRC-5)
  - Produces characteristic CPE



Negative fibroblast Cell line

HSV CPE. consists of cell rounding starting on the edge of the monolayer.



Histology/Cytology – Observe for multinucleated giant cells, cannot differentiate HSV 1, 2, and VZV

Molecular Amplification -Standard of practice for detecting HSV 1/2 from lesions and CSF

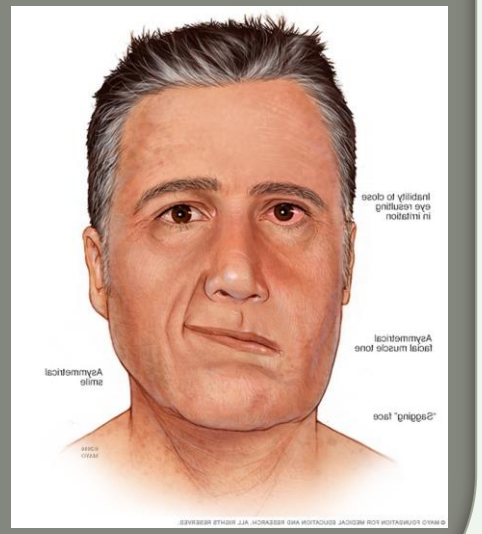
Serology – used to screen for past infection, not for acute diagnosis

Varicella Zoster Virus

- Transmission: close contact
- Latency: dorsal root ganglia

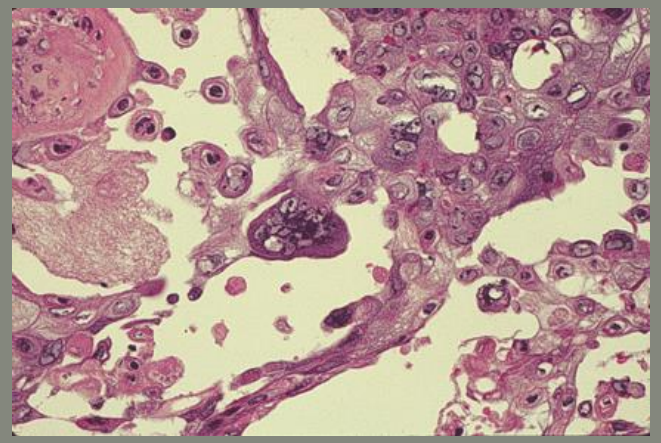
- Diseases:
  - Chickenpox (varicella)
    - More serious disease in adults and immune suppressed with possible progression to pneumonia and/or encephalitis

  - Shingles (zoster – latent form of VZV) lesions appear over isolated dermatomes
  - Ramsay-Hunt syndrome – facial nerve infected with facial paralysis



Varicella Zoster Virus Diagnosis

- Histology – multi-nucleated giant cells formed
- Serology – usually for immune status check
- Cell culture – growth in 5-7 days, fibroblasts
  - Sandpaper like appearance in cell monolayer with scattering of rounded cells
- Molecular Amplification for disease diagnosis
- Effective vaccine has lowered the incidence of VZV in children and shingles in adults



Cytomegalovirus (CMV)

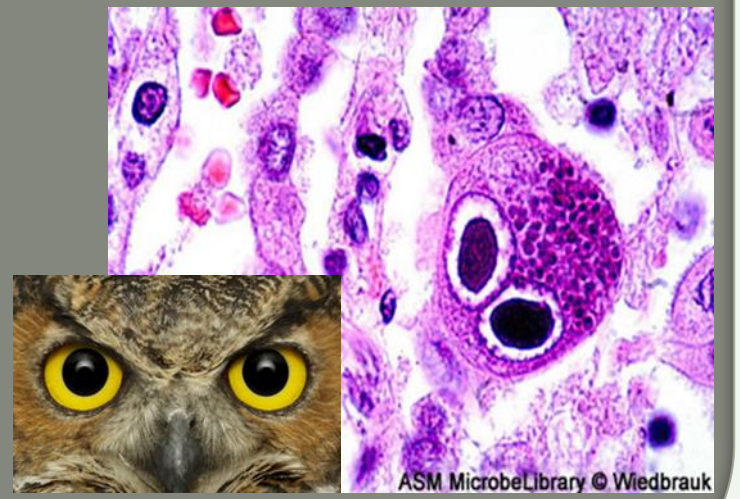
- Transmitted by blood transfusion , vertical and horizontal transmission to fetus, close human contact, and sexual contact
- Latency: Macrophages
- Disease
  - Initial infection asymptomatic in most and occurs in the first decade of life
  - Congenital – most common cause of TOR**C**H infection
  - Perinatal acquisition of infection

CMV Infection

- Mononucleosis – Fever, no swollen lymph nodes, heterophile antibody test is negative
- Organ specific diseases:
  - Gastrointestinal
  - Hepatic
  - Neurological
  - Cardiovascular
- Primary infection in the immune suppressed host is more serious than recurrent or secondary infection

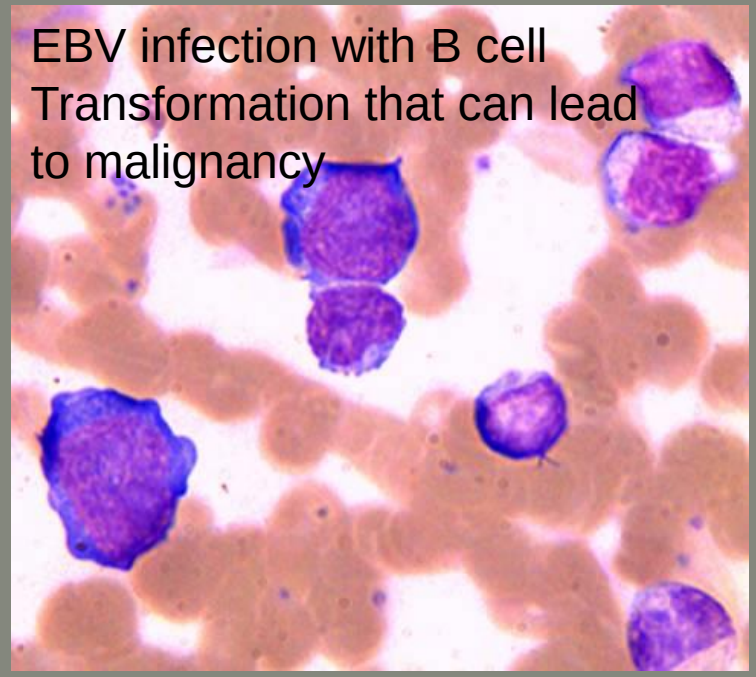
CMV Diagnosis

- Laboratory Diagnosis:
  - Cell culture using Human diploid fibroblast cells
    - Cytopathic effect can take >=14 days
    - CPE described as grape like clusters of rounded cells
  - Quantitative PCR is very useful
    - Due to persistent CMV shedding in most infections quantitative PCR used to detect significant viral loads, most consistent with ongoing infection
  - Histopathology – Infects epithelial cells, macrophages and T lymphocytes. Intranuclear and intracytoplasmic inclusions (Owl eye)
- Treatment: ganciclovir, foscarnet, cidofovir



Epstein Barr virus (EBV)

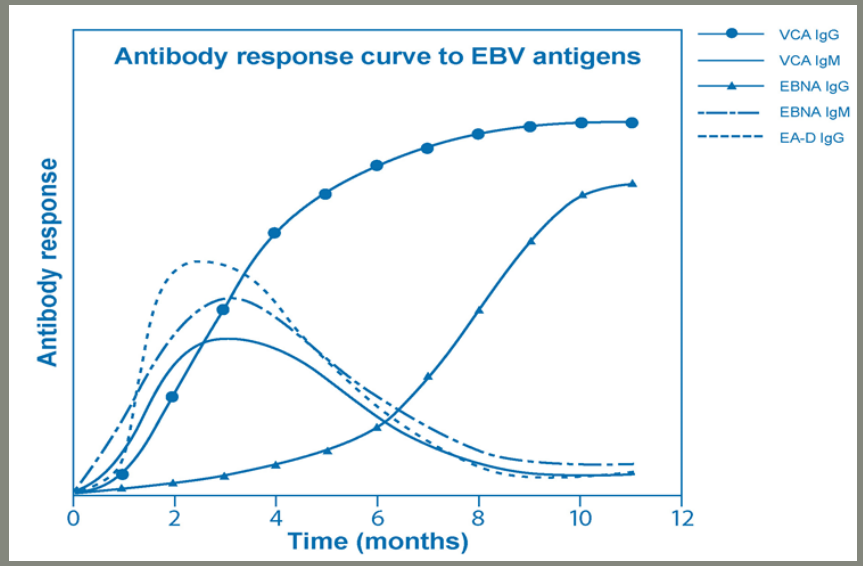
- Transmission - close contact, saliva
- Latency in the B lymphocyte
- CD21 cell receptor for lymphocyte invasion
- Diseases include
  - Infectious mononucleosis
    - Heterophile antibody produced
    - Patient serum reacts with horse and cattle rbc
  - Lymphoreticular disorders
  - Oral hairy leukoplakia
  - Burkitt's lymphoma
  - Nasopharyngeal Carcinoma
  - 1/3 of Hodgkin's lymphoma cases
- Will not grow in viral cell culture
- Serology and molecular methods for diagnosis



Serologic Diagnosis of EBV

VCA IgM	VCA IgG	EBNA-1 IgG	
Negative	Negative	Negative	No immunity
Positive	Negative	Negative	Acute infection
Positive	Positive	Negative	Acute infection
Negative	Positive	Positive	Past infection
Negative	Positive	Negative	Acute or past infection
Positive	Positive	Positive	Late primary infection
Negative	Negative	Positive	Past infection

VCA = viral capsid antibody
EBNA = EBV nuclear antigen



Human Herpes virus types 6 & 8

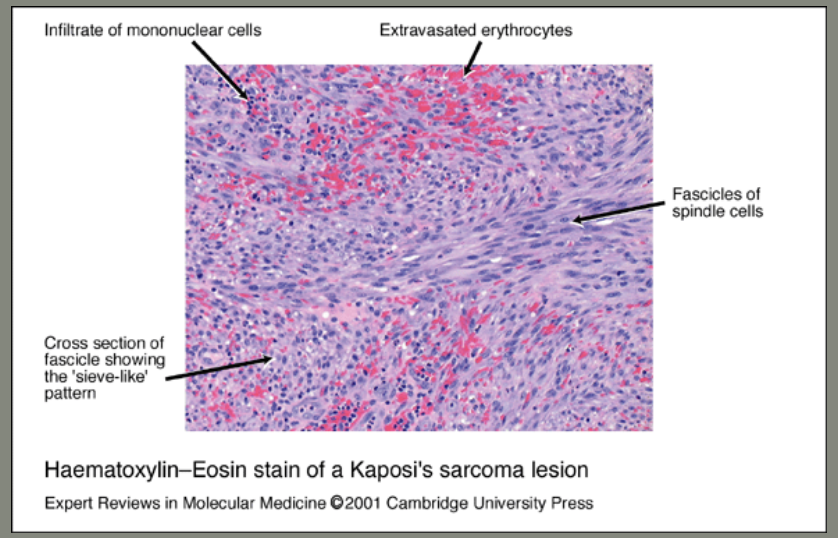
HHV-6

- Children:
  - Roseola [sixth disease] usual age 6m-2yr, high fever & rash

- Adult
  - In immune compromised can cause encephalitis
    - Can also undergo germ line integration and be a source of false positive results in the normal host when testing CSF, must be cautious in test result interpretation

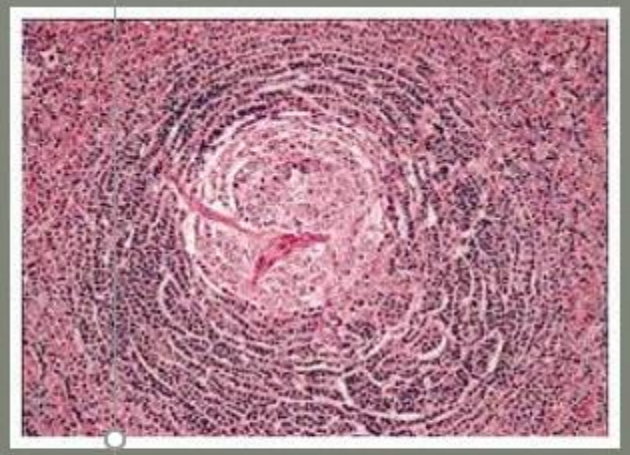
HHV-8

- (1) Kaposi's sarcoma



HHV- 8

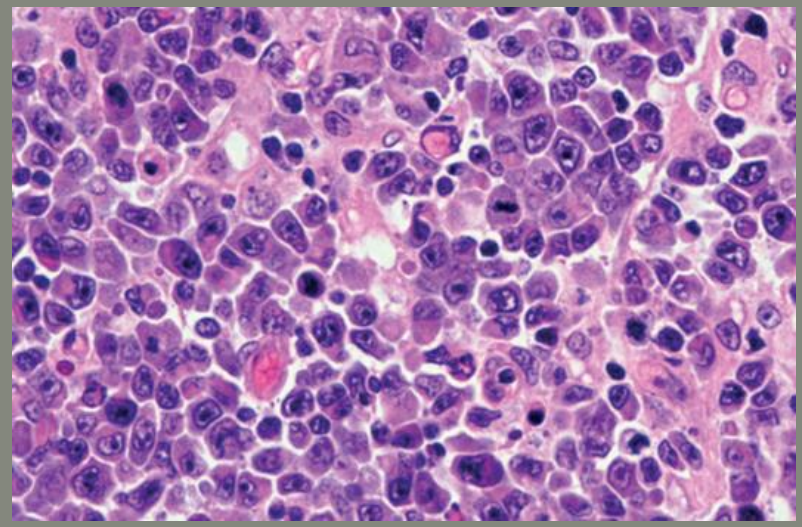
- Castleman disease

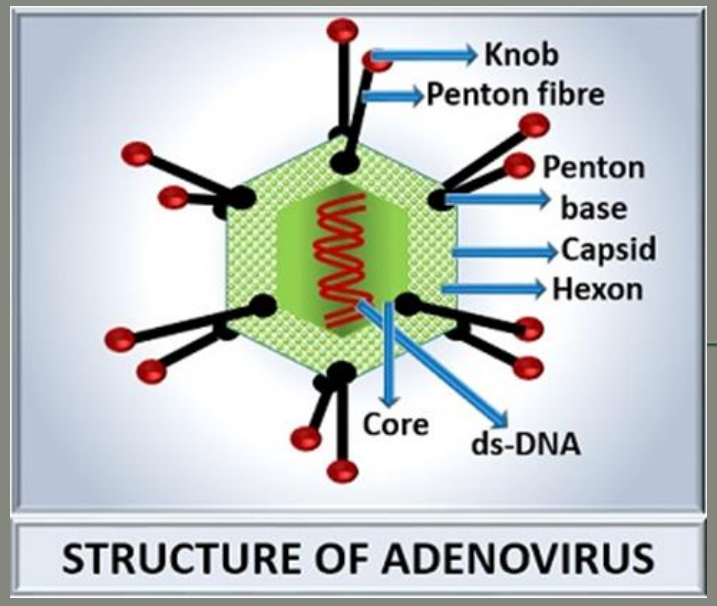


Onion skin pattern of Castleman disease – lymph node

- Primary effusion lymphoma
  - Most often in HIV/AIDS patients
  - Localized in body cavities without a tumor mass

    Large cells , large round to irregular nuclei and prominent nucleoli





Adenoviridae

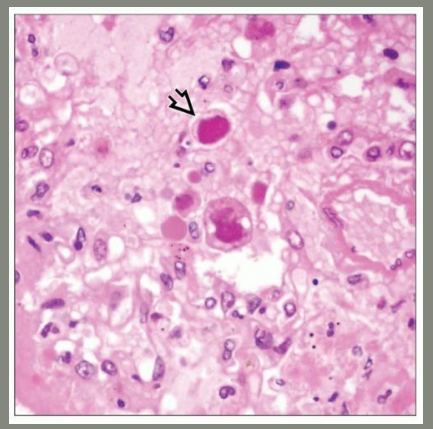
Adenovirus

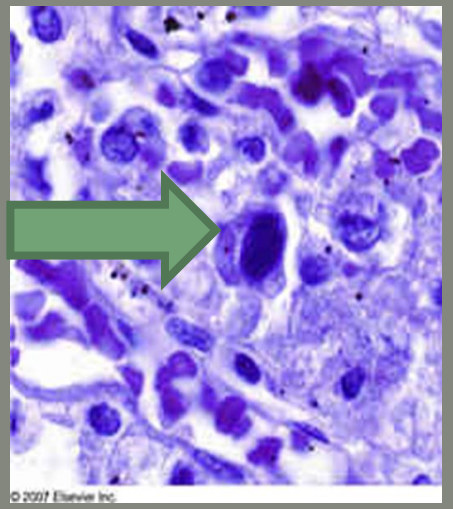
Adenovirus

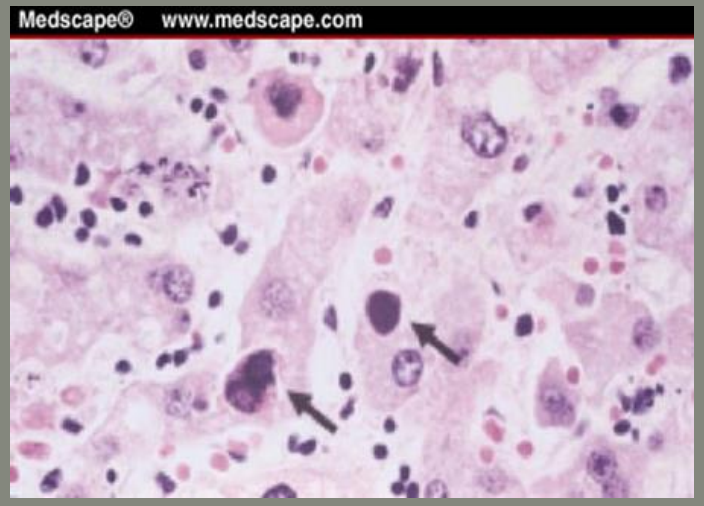
- DNA - non enveloped/ icosahedral virus
- Latent: lymphoid tissue
- Transmission: Respiratory and fecal-oral route
- Diseases:
  - Pharyngitis (year-round epidemics)
  - Pneumonia
  - Gastroenteritis in children
    - Adenovirus types 40 & 41
  - Kerato-conjunctivitis – usually bilateral red painful and inflamed eyes for @ 2 weeks
  - Disseminated infection in immunosuppressed – often starts with pneumonia, @ 75% fatality/ no therapy
  - Hemorrhagic cystitis in immune suppressed/particularly in transplantation

Adenovirus

- Diagnosis
  - Cell culture (CPE), <=5 days
  - Molecular methods (PCR) superior
  - Stool antigen detection by EIA (40/41 strains)
  - Supportive treatment only – no specific viral therapy
  - Histology - Intranuclear inclusions / smudge cells – Basophilic and nuclear membranes become blurred



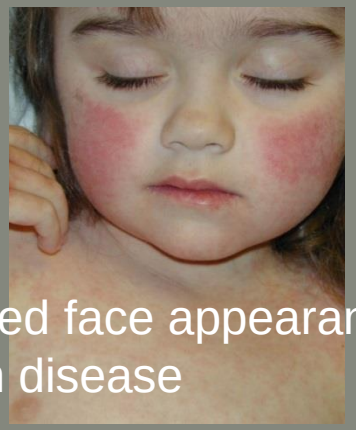




Parvoviridae

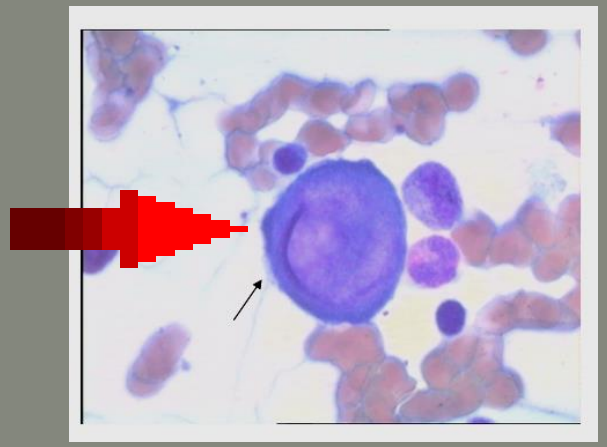
Parvovirus

Parvovirus B19



Slapped face appearance of fifth disease

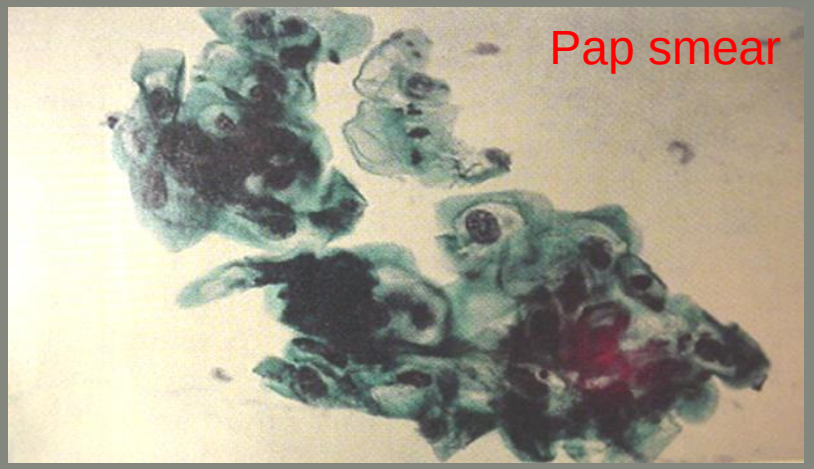
- DNA virus infects humans
- Infections:
  - Erythema infectiosum (Fifth disease) – infection with headache rash and cold-like symptoms in young children
  - Hydrops fetalis – infection in pregnant, particularly infection in 1st trimester can lead to miscarriage
  - Aplastic crisis in patients with pre-existing bone marrow stress
  - Chronic hemolytic anemia in patients with HIV/AIDS
  - Arthritis and Arthralgia
- Histology - Virus infects mitotically active erythroid precursor cells detected in bone marrow
- Molecular and Serologic methods to confirm histologic diagnosis



Papovaviridae

Papillomavirus

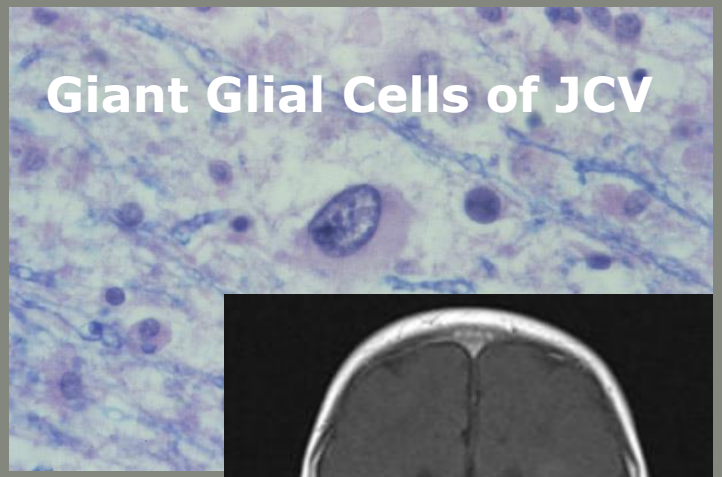
Papillomavirus

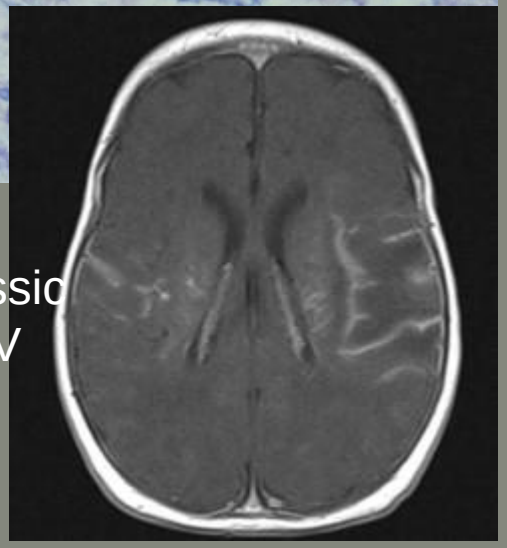


- Diseases:
  - Skin and anogenital warts
  - Benign head and neck tumors
  - Cervical and anal intraepithelial neoplasia and cancers
    - High risk HPV types 16, 18 cause @ 70% of cervical cancers.
    - Other high-risk types include: 31, 33, 45, 53, 58
    - Low risk HPV types 6 and 11 cause @ 90% Genital warts
- Diagnosis :
  - Pap Smear
  - Molecular methods: Detection and typing of HPV types
  - Guidelines suggest both PAP and molecular HPV testing for women 30 - 65 years of age / performed every 5 years
  - Vaccines - 1°to guard against HPV 6,11,16,18 for young females and males

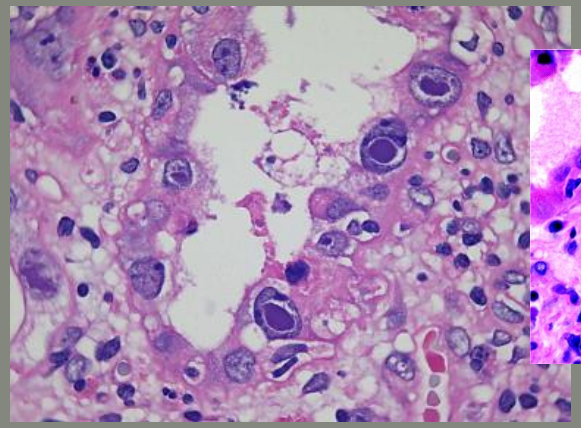
Polyomavirus

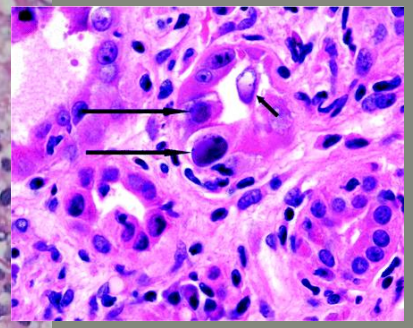
- JC virus
  - Progressive multifocal leukoencephalopathy (PML)
    - Encephalitis of immune suppressed
    - AIDS, cancer and immune suppression
    - Diagnosis:
    - Destroys oligodendrocytes in brain with formation of giant glial cells
- BK virus
  - Latent virus infection in kidney / Nephropathy
  - Progression in immune suppression
  - Hemorrhagic cystitis
  - Diagnosis:
  - Histology/ homogenous and purple intranuclear
  - inclusions, primarily in tubular epithelium





Demyelination is classic finding on MRI in JCV





Hepadnaviridae

Hepatitis B

Hepatitis B

- Enveloped DNA virus
- Child can acquire from mother during birthing process
- Spread by contact with blood and other body fluids
- Spectrum of Hepatitis B symptomatology
  - Acute phase - disease varies from subclinical hepatitis to icteric hepatitis fulminant, acute, and subacute hepatitis
  - Chronic phase - chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC)
- Vaccinate to prevent
- Therapies available as long as minimal liver damage
- Diagnosis
  - Serology and Molecular assays

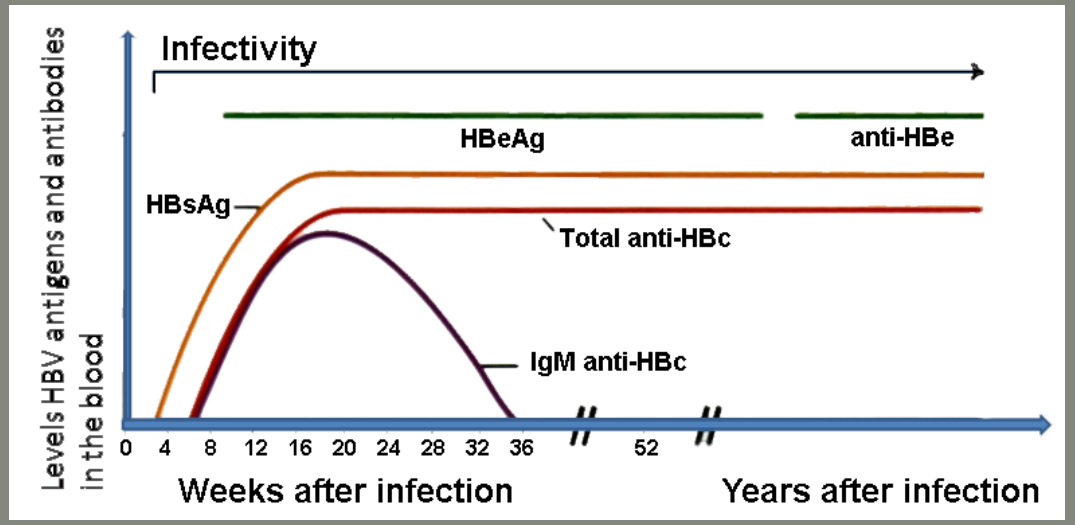
Hepatitis B Serology

- **Surface Antigen Positive**
  - Patient has Active Hepatitis B or is a Chronic Carrier
    - Next perform Hep B Quantitation for to assess viral load
    - Perform Hep e antigen test – if positive, patient is a chronic carrier and has a worse prognosis

- **Core Antibody Positive**
  - Immune due to prior infection, acute infection or chronic carrier

Surface Antibody Positive

- Immune due to prior infection or vaccine



Flaviviridae

Hepatitis C

Mosquito borne Flaviviridae:

Dengue

Zika

Yellow fever

West Nile

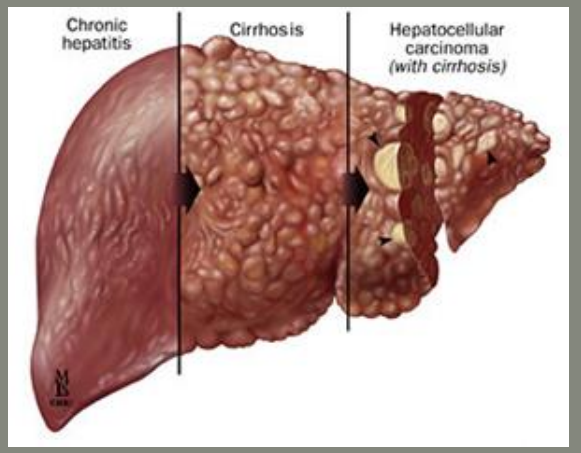
Tick borne Flaviviridae

Powassan fever

Hepatitis C Virus

- Disease acquisition:
  - Parenteral transmission, drug abuse, sexual, blood products or organ transplants (prior to 1992), poorly sterilized medical equipment
- Infection of humans and chimpanzees
- 3.2 mil persons in US have/had chronic Hep C
- Seven major genotypes (1-7)
  - Acute self-limited disease that progresses to a disease that mainly affects the liver
  - Type 1 virus most common in USA
  - Infection persists in @ 75-85% without symptoms
  - 5 - 20 % develop liver cirrhosis
  - 1-5 % develop hepatocellular carcinoma

Hepatitis C



- Diagnosis:
  - Hepatitis C IgG antibody test is positive
  - Must then perform:
    - RNA quantitative assay for viral load
    - Genotype of virus for proper therapy selection and duration
    - Assessment of liver damage
- No vaccine available
- Antivirals currently FDA cleared that can cure >= 85% of patients infected with Hepatitis C
  - Therapy can not reverse cirrhosis

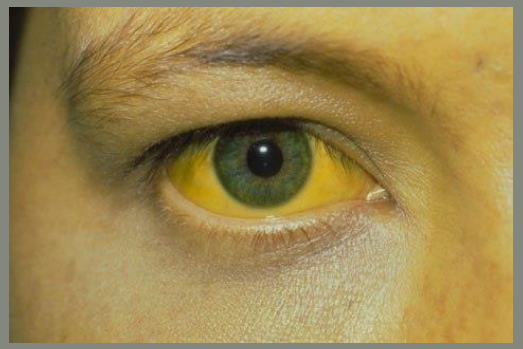
Flavivirus – Mosquito borne

- Dengue – "break bone fever"
- Vector: *Aedes aegypti* mosquito / found in Asia and the Pacific
- Disease
  - Fever, rash, and severe joint pain
  - Small % progress to a **hemorrhagic fever which can be fatal**
- Diagnosis: Serology / IgM for acute infection

- Zika virus
  - Vector: *Aedes aegypti* and *A. albopictus* mosquitoes
  - Outbreak 2016 in South America (Brazil) and spread to central America, Caribbean and US (Miami)
  - Milder disease than Dengue in most adults, with fever and rash
  - Neurologic tropism makes it more problematic
    - Microcephaly in fetuses borne to infected moms
    - Potential developmental issues can occur in infected children
    - Guillain-Barre syndrome sequelae in adults
  - Diagnosis: Serum IgM / Molecular assays serum, urine, amniotic fluid and CSF

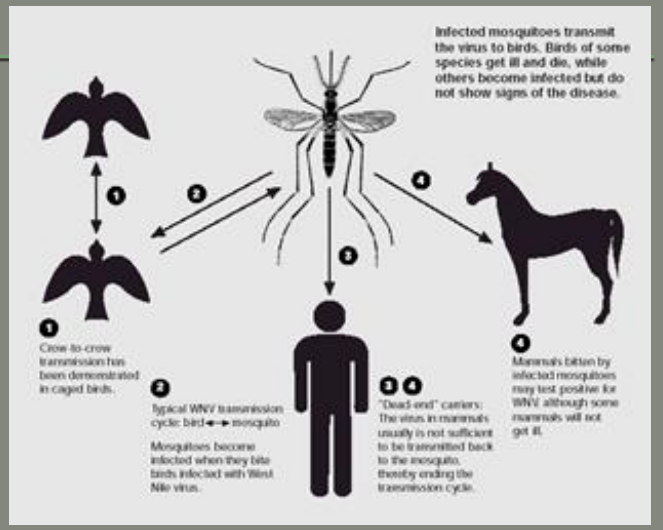
Flavivirus – Mosquito borne

- Yellow fever
- Vector – *Aedes aegypti* mosquito
- Outbreak Brazil 2018 /large numbers of infected mosquitoes introduced into a heavily populated Brazilian cities
- Endemic in Africa, Central and South America
- Most cases mild with 3-4 days fever, headache, chills, back pain, fatigue, nausea, vomiting
  - 15% experience liver damage (jaundice) and hemorrhagic issues (20 – 50% fatality rate)
- Diagnosis:
  - Molecular testing for virus in serum
- No specific anti-viral drugs for therapy
- Vaccine – supplies life-long immunity



Flavivirus – Mosquito borne

- West Nile
  - Vectors: *Culex* mosquito
  - Common across the US
  - Birds primary reservoir, humans and horses at risk
  - Disease
    - 80% asymptomatic
    - 20% fever, headache, muscle weakness
    - Small % progress to encephalitis, meningitis, flaccid paralysis
    - Serology and Molecular assays
      - Molecular testing for West Nile has low sensitivity, and narrow time range for detection, both methods are necessary for diagnosis



Cross reactions occur in serologic testing for the Flavivirus family: Dengue, Zika and West Nile virus, present diagnostic problems/ Public health laboratories assist

Flavivirus– Tick borne

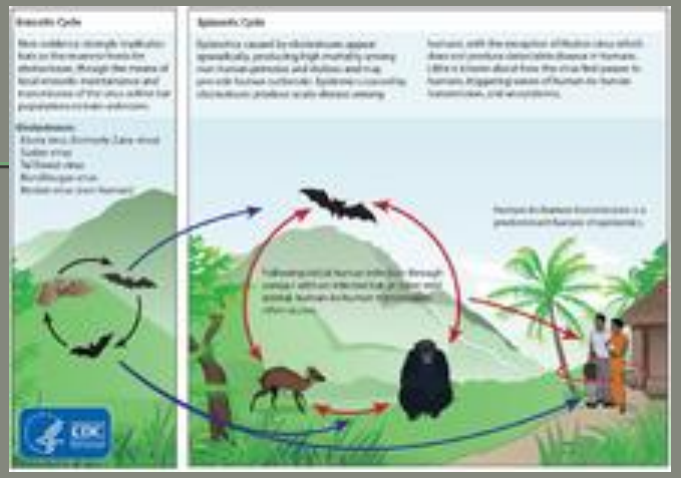
- Powassan (POW) virus
- Vector: *Ixodes* ticks
- Approximately 100 cases of POW virus disease were reported in the United States over the past 10 years.
  - Northeast and Great Lakes region of US
- Fever, headache, vomiting, weakness, confusion, seizures, and memory loss, can cause significant swelling in the brain
- Long-term neurologic problems may occur.
- There is no specific treatment

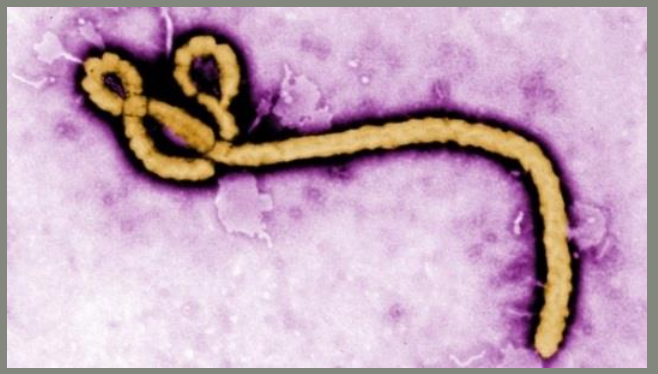
Alpha virus- mosquito borne

- Chikungunya virus
  - Vector: Aedes mosquito including aegypti
  - Origin in Asia and African continents with recent migration to the Caribbean and SE USA
  - Acute febrile illness with rash followed by extreme joint pain,
  - No hemorrhagic phase
- When screening for ZIKA – need to rule out infection with Dengue and Chikungunya. Similar diseases with very different sequelae/ public health departments assist

Ebola Virus

- >20 outbreaks since discovery in 1976
  - Most recent began in Dec 2013 - West Africa
  - Prolonged outbreak due to area affected has high population with limited medical resources
- Transmission from direct contact with bodily fluids – fatality rate 55%
  - Animal reservoir: fruit bats
- Asymptomatic are not contagious
- Fever, weakness, myalgia, and headache
  - Consider malaria and typhoid in the differential
  - Multifocal necrosis in liver, spleen, kidneys, testes and ovaries
- Susceptible to hospital disinfectants
- Testing at CDC (EIA, PCR), detectable at >= 4 days of illness
- Level A agent of Bioterrorism





Coronavirus

Middle East Respiratory Syndrome (MERS)

- Endemic in Arabian peninsula (2012)
- Direct contact with infected camels
- Close human to human contact can spread infection –
- no outbreaks – 30% fatality rate from respiratory failure
- Fever, rhinorrhea, cough, malaise followed by shortness of breath

Severe Acute Respiratory Syndrome 1 (SARS)

- Outbreak in China 2003 – spread to 29 countries
- Dry cough and/or shortness of breath with development of pneumonia by day 7-10 of illness
- Laboratory testing public health laboratories (CDC) -antibody testing enzyme immunoassay (EIA) and molecular tests for NP, Throat, sputum, blood, and stool specimens.

Coronavirus Disease 2019 (COVID)

- SARS-CoV-2
  - Bat Coronavirus
  - Introduced into human population in open animal market in Wuhan, China (12/2019)
  - Spread primarily by respiratory droplet
  - Influenza-like illness ranging from asymptomatic and mild symptoms to severe respiratory illness and systemic complications and death (death rate @ 2 .5%):
    - Fever, dry cough, shortness of breath, sore throat, nasal congestion, loss of sense of smell and taste, and diarrhea
  - Diagnosis:
    - Collection of nasopharyngeal or mid-turbinate nasal
    - Detection of SARS CoV-2 viral RNA by molecular and antigen assays
    - Antibody detection (IgM and IgG) mostly used to detect past infection
  - Vaccine: currently clearing regulatory review

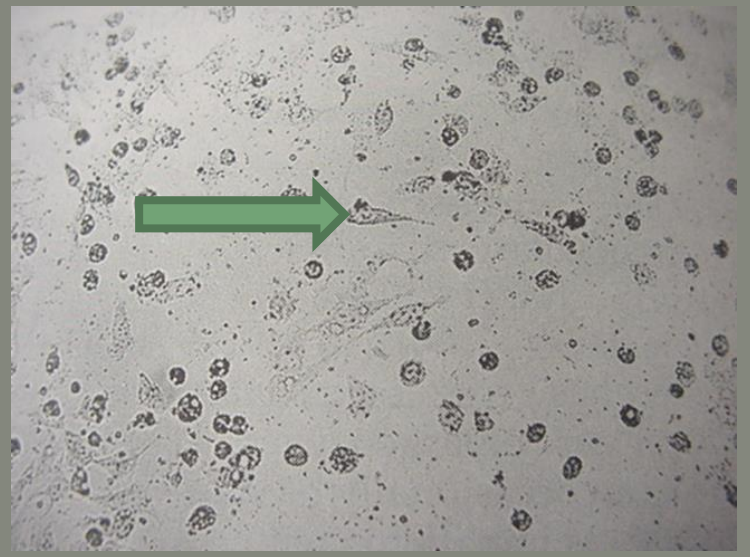
Picornaviridae

Enterovirus

Hepatitis A

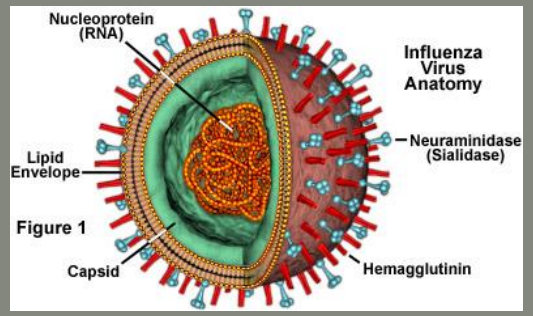
Enteroviruses

- Diverse group of > 60 viruses – SS RNA
- Seasonal viruses most often in summer and fall
  - Polio virus/ paralysis
    - Salk Inactive Polio Vaccine (IPV) recommended
  - Coxsackie A /vesicular oral lesion (Herpangina)
  - Coxsackie B/ Pericarditis/Myocarditis
  - Enterovirus /Aseptic meningitis in children, hemorrhagic conjunctivitis, Acute flaccid myelitis EV-D68
  - Echovirus /various infections, intestine
  - Rhinoviruses /common cold
- Grow in continuous cell lines in 5-7 days
  - Teardrop or kite like cells formed
- Molecular assays superior for diagnosis



Hepatitis A

- Fecal – oral transmission, contaminated food or person to person
- Common traveler's disease
- Recent outbreaks in homeless populations due to inadequate sanitary facilities
- 80% infected develop symptoms – jaundice & elevated aminotransferases
- Incubation 15- 50 days with abrupt onset, low mortality, no carrier state
- Diagnosis – serology with IgM positive in early infection
- Antibody is protective and lasts for life
- Vaccine available



Orthomyxoviruses
Influenza A

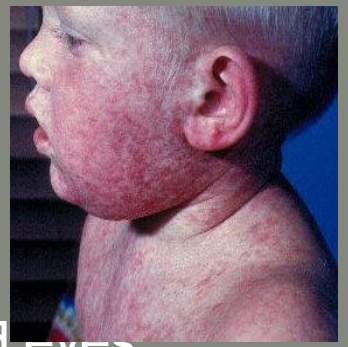
- Hemagglutinin (H) and Neuraminidase (N) are glycoprotein spikes on outside of influenza viral capsid
  - H and N provide typing of virus strains – such as H1N1 and H3N2
- **Antigenic drift** - minor change in the amino acids of either the H or N glycoprotein
  - Cross antibody protection will still exist so an epidemic will not occur
- **Antigenic shift -** genome reassortment with a "new" virus created/usually from bird or animal/ this could create a pandemic
  - H5N1 = Avian Influenza
  - H1N1 = 2009 Influenza A

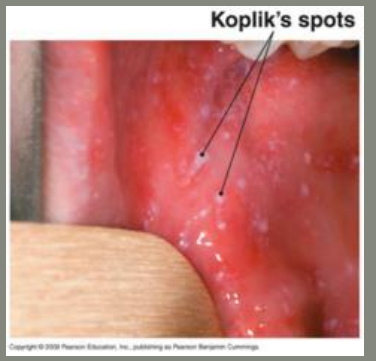
Influenza A

- Disease: Acute onset of respiratory symptoms (nose, throat or lung) which can progress to secondary bacterial lung infection
- Yearly H and N types dominate, most recently H1N1 and H3N2
- Diagnosis
  - Cell culture obsolete / no characteristic CPE
  - Enzyme immunoassay (EIA) lateral flow membrane used in point of care testing
  - Amplification (PCR) gold standard for influenza detection
- Treatment: Amantadine and Tamiflu (Oseltamivir)
  - Seasonal variation in susceptibility but Tamiflu has remained sensitive
- Influenza B
  - Milder form of Influenza illness with similar respiratory symptoms to influenza A
  - Usually <=10% of cases of influenza with 90% caused by Influnzae A
- Vaccinate – Quadrivalent vaccine that contains 2 A types and 2 B types

Paramyxoviruses

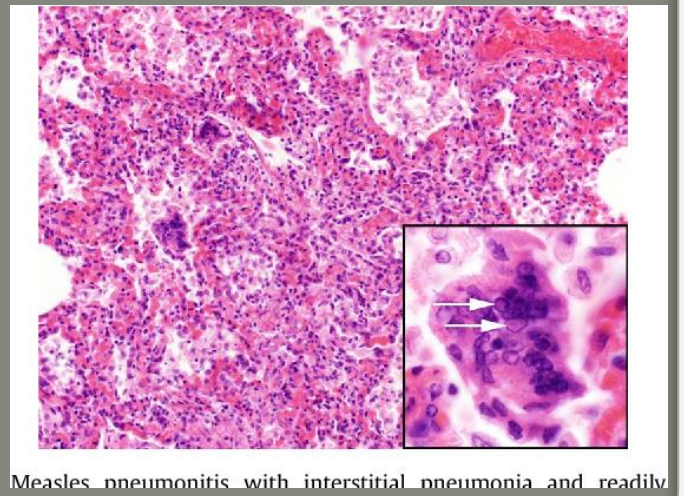
Measles





- Disease
  - Fever, Rash, Dry Cough, Runny Nose, Sore throat, inflamed eyes (photosensitive),
  - Very contagious viral illness
  - Koplik's spot – small red spots with central bluish discoloration- seen in the inner lining of the cheek
  - Subacute sclerosing panencephalitis [SSPE]
    - Rare chronic degenerative neurological disease
    - Persistent infection with a mutated measles virus, due to mutated virus there is total lack of an immune response

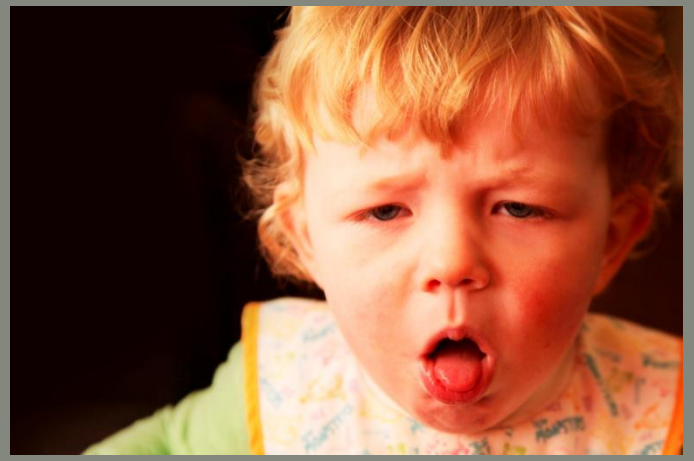
H and E stain/ lung



Measles pneumonitis with interstitial pneumonia and readily

- Diagnosis: Clinical symptoms, Molecular tests collected from nasal or throat is, IgM serology can have false positive reactions
- Histology for acute lung injury – multinucleated giant cells, inclusions with perinuclear halos
- Vaccinate – MMR (Measles, Mumps, Rubella) vaccine
- Treatment: Not specific, Immune globulin, vitamin A

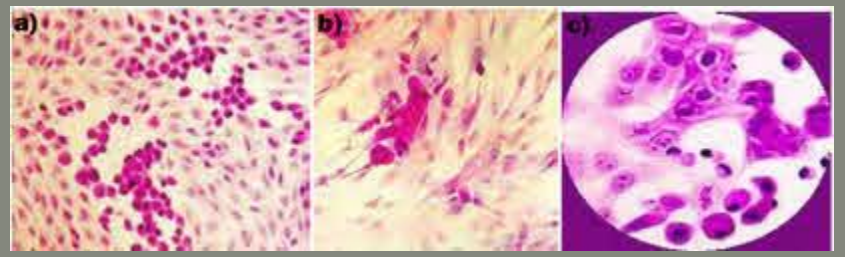
Parainfluenza



- Types 1, 2, 3, and 4
- Person to person spread
- Disease:
  - Upper respiratory tract infection in adults and children with fever, runny nose and cough
  - Lower respiratory tract infection - Croup, bronchiolitis and pneumonia more likely in children, elderly and immune suppressed
- Molecular methods standard of practice
- Supportive therapy only available
- No vaccine

Mumps

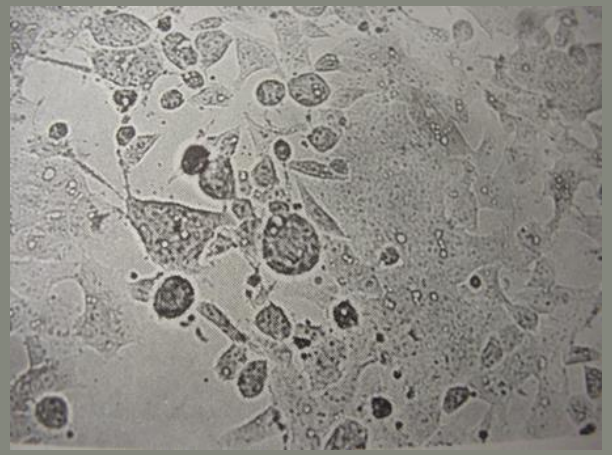
- Person to person contact
- Leads to Parotitis, other sites affected less commonly: testes/ovaries, Eye, Inner ear, CNS
- Diagnosis: clinical symptoms ,serologic tests, and molecular assays
- In cell culture multinucleated cells formed
- Histology – mixed inflammatory cells and necrosis
- Prevention: Measles/Mumps/Rubella (MMR) vaccine
- No specific therapy, supportive



Mumps Orchitis

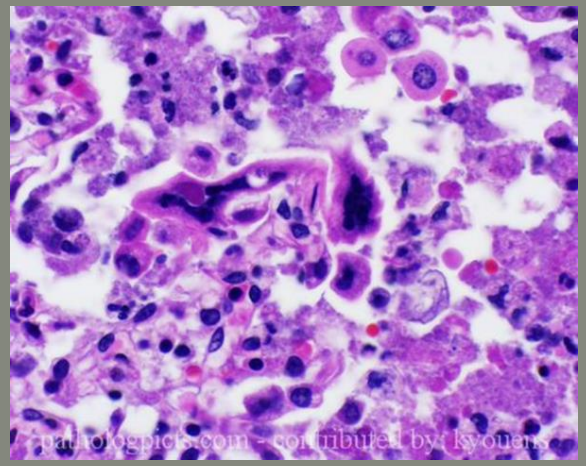
Respiratory Syncytial Virus (RSV)

- Respiratory disease - common cold to pneumonia, bronchiolitis to croup (young infant) more serious in infants and immune suppressed
  - Transmission by contact and respiratory droplet
- Diagnosis: Antigen detection by EIA cell, Cell culture in Hep-2 or HeLa cell lines, Molecular testing (best) and lung biopsy
- Treatment: Ribavirin



Classic CPE = Syncytium formation(multinucleated giant cell formation) in HeLa cell line

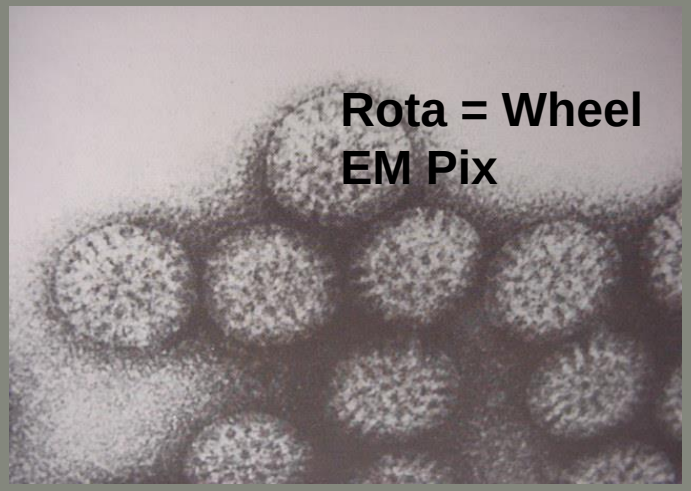
Syncytium formation
In lung tissue



Human Metapneumovirus

- 1st discovered in 2001 – seasonal (winter) community acquired viral respiratory tract disease
  - 95% of cases in children <6 years of age but can be seen in the elderly and immune suppressed
  - Upper and lower respiratory tract disease
    - 2nd only to RSV in the cause of bronchiolitis
- Will not grow in viral cell culture
- Molecular assays (PCR) for diagnosis
- Treatment: Supportive, no specific anti-viral therapy available

Reoviridae
Rotavirus



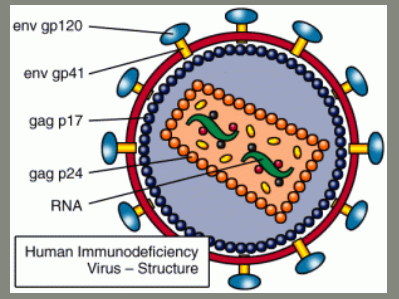
- Winter - spring seasonality
  - Gastroenteritis with vomiting – most common cause of severe diarrhea in children 6m – 2 yr
  - Fecal – oral spread
- Major cause of childhood death in 3rd world countries due to fluid loss in the small child
- Diagnosis – unable to grow in cell culture
  - Antigen detection in stool using Enzyme immunoassay (EIA) and molecular assays
- Vaccine available which has greatly decreased infections

Caliciviruses
Norovirus

- Spread by contaminated food and water, feces & vomitus –
- Only requires <=20 virus particles to spread infection
- Tagged the "Cruise line virus" due to numerous outbreaks aboard cruise liners, but there are many other sites with outbreaks
- Leading cause of epidemic gastroenteritis in all age groups
  - Fluid loss from vomiting and diarrhea quite debilitating, especially children
- Disease course usually 24-48 hours
- Diagnosis: Molecular methods testing stool
  - Will not grow in viral cell culture

Retrovirus

Human Immunodeficiency virus



- **CD4 primary receptor site on lymphocyte** for attachment and entry of virus
- **Reverse transcriptase enzyme** converts genomic RNA into DNA
- Transmission - sexual, blood and blood product exposure, perinatal
- Non-infectious complications of HIV/AIDS:
  - Lymphoma, Kaposi's sarcoma, anal cell carcinoma, and non Hodgkin's lymphoma
- Infectious complications:
  - Pneumocystis, Cryptococcal meningitis, TB and Mycobacterium avium complex, Microsporidia, Cryptosporidium, STD's , Hepatitis B and C

HIV Laboratory Diagnosis

Antibody Enzyme immunoassay with Western Blot confirmation (**old way**)

- Positive EIA tests confirmed by a Western blot test
  - Western blot detects gp160/gp120 (envelope proteins), p 24 (core), and p41(reverse transcriptase)
  - Must have at least 2 solid bands on Western blot to confirm as a positive result for HIV disease

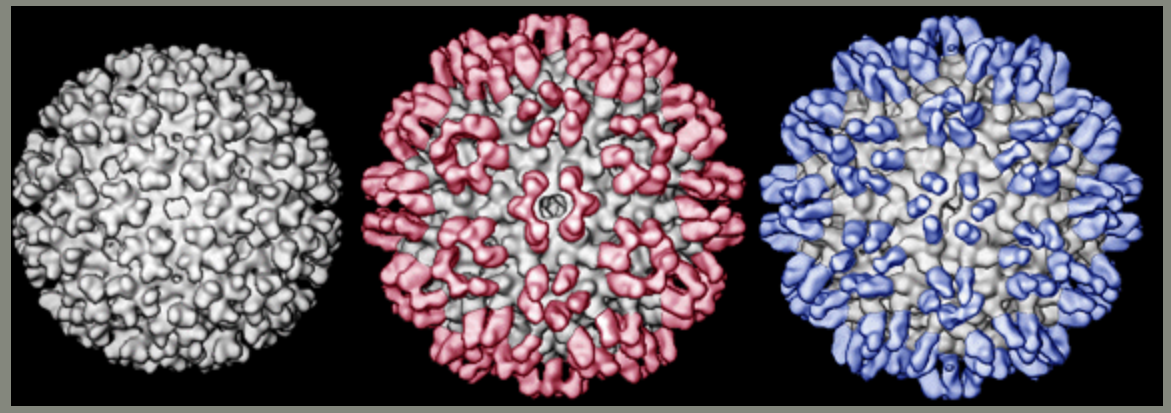
Newer generation test - Antigen/antibody combination (4th generation) immunoassay* that detects IgG and IgM HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen to screen for established and acute infection

Detects HIV infection earlier @ 2- 4 weeks

Positive patients require additional testing:

- HIV viral load quantitation, is there >= 100 copies of virus present
- Resistance gene testing – report viral subtype to optimize therapy
  - Most isolates in USA type B
- Monitor for low CD4 counts for HIV infection severity

Togaviridae



RNA Virus

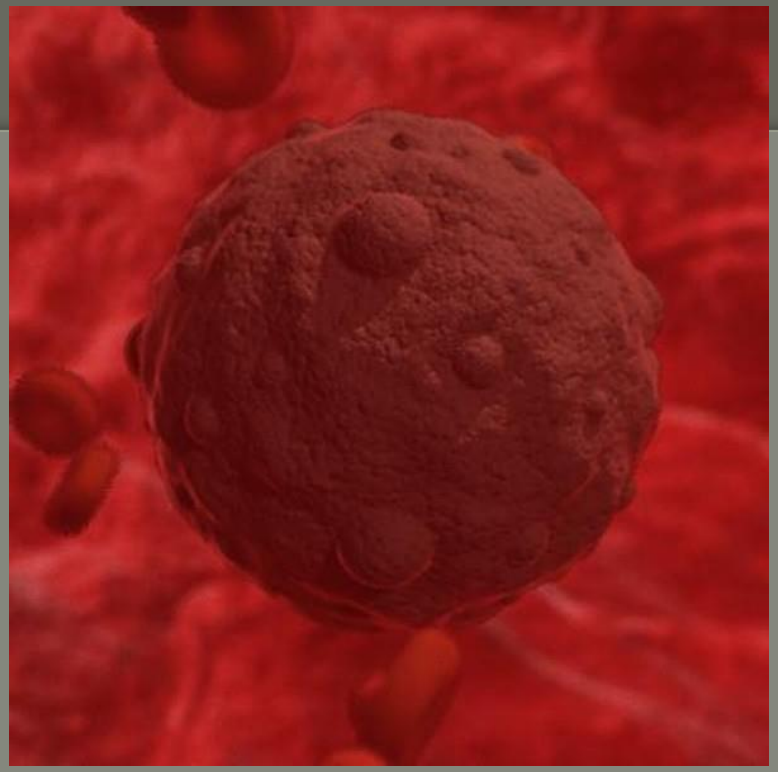
Rubella

Togaviridae
Rubella

- "Three day measles" or German measles
- Relatively mild disease with rash, low grade fever, cervical lymphadenopathy
- Respiratory transmission
- Congenital rubella –
  - Occurs in a developing fetus of a pregnant women who has contracted Rubella,
  - Highest % (50%) in the first trimester of pregnancy
  - Prior to Zika virus, it was the neurotropic virus of the fetus
    - Infection caused deafness, eye abnormalities, congenital heart disease
- Diagnosis: serology in combination with clinical symptoms
- Live attenuated vaccine (MMR) to prevent

Bunyaviridae

enveloped RNA viruses



Hantavirus

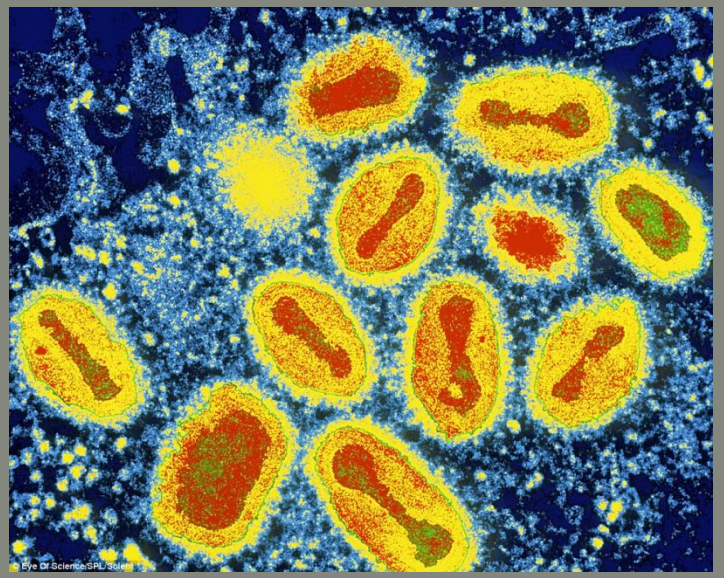
Bunyaviridae
Hantavirus

- Outbreak occurred on Indian reservation in the four corner states (NM,AZ,CO,UT) 1993 / led to description of this disease
- Outbreak Yosemite National Park cabins (2012)
- Transmission
  - Urine and secretions of wild deer mouse and cotton rat
  - Outbreak from transmission from pet rats to humans
- Infected animals found in states west of the Mississippi River
- Myalgia, headache, cough and respiratory failure
- Diagnosis: Serology
- Supportive therapy

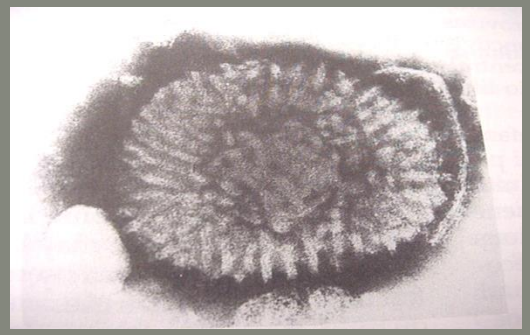
Poxviruses

Smallpox virus

Variola virus
Vaccinia virus



Poxvirus
Smallpox



- Variola virus – agent of Smallpox, eradicated in 1977
- Vaccinia virus - active constituent in the Smallpox vaccine, virus is immunologically related to smallpox
  - Vaccinia can also cause disease mostly in immune suppressed, which would prevent vaccination of this population to smallpox
- Disease begins as maculopapular rash progressing to vesicular rash / rash moves from initial lesions on central body outward
- BSL4 conditions required for laboratory work
- Reported to public health for case investigation and molecular diagnostic testing

Rhabdoviruses

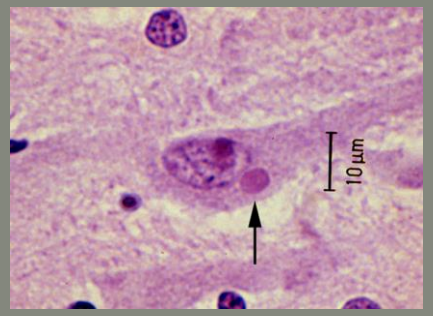
bullet shaped RNA virus

Rabies virus

Rhabdoviruses
Rabies

- Worldwide in animal populations
  - Bat and raccoon primary reservoirs in US
  - Dogs reservoir in 3rd world countries
- Infections from bites and inhalation of aerosolized saliva, urine and feces
- Post exposure rabies vaccine and rabies immunoglobulin PRIOR to the development of symptoms prevent disease development
- Classic disease symptom is excessive salivation from paralysis of throat muscles
- Diagnosis: Detection of viral particles (Negri bodies) in the brain and
- detection of rabies genetic material using molecular assays in saliva
- Public health department should be contacted to assist with diagnosis

Intracytoplasmic
Negri bodies
In brain biopsy



Bullet
shaped viral
particles

Creutzfield-Jacob Disease (CJD)

- Family of rare degenerative fatal brain disorders known as
- Transmissible spongiform encephalopathies (TSE) so named due to the microscopic appearance of the infected brain
  - Spongiform change in the gray matter observed on stained brain biopsy
  - Biopsy is most definitive test for diagnosis
- A Protein prion thought to cause this disorder
- Protein product 14-3-3 can be tested for in CSF
  - Biproduct of cell death in the brain
  - Not specific for CJD

- Safety important to prevent transmission
  - Universal Precautions
  - Use disposable equipment when possible