Virology Course

For Microbiology Diploma Students

Prepared by: Dr. Asmaa Sabry Yassein

Ass. Prof. of Microbiology

رؤية الكلية:

التميز في تعليم العلوم الأساسية والبحث العلمى للمساهمة في التنمية المستدامة.

رسالة الكلية:

تقديم تعليم مميز في مجالات العلوم الأساسية وإنتاج بحوث علمية تطبيقية للمساهمة في التنمية المستدامة من خلال اعداد خريجين متميزين طبقا للمعايير الاكاديمية القومية وتوفيرخدمات مجتمعية وبيئية تلبى طموحات جنوب الوادى وبناء الشراكات المجتمعية الفاعلة.

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General introduction

Virology: is the scientific discipline concerned with the study of the biology of viruses and viral diseases, including the distribution, biochemistry, physiology, molecular biology, ecology, evolution and clinical aspects of viruses.

Viruses: are infective agent consists of nucleic acid in a protein coat

Too small to be seen by light microscopy or can be defined as simple, non-cellular, very small sub-microscopic biological entities consisting of one or more molecules of either DNA or RNA

Multiple within the living cell (host) so they are obligate parasite

Smallpox is the largest virus about 200 nm and polio virus is the smallest virus about 28 mm in diameter.

Viruses vary in shape from the simple helical and icosahedral to more complex structures.

Classification of Viruses

1- DNA Viruses

a- dsDNA includes viruses:

Hepadna

Herpes

Adeno

Papo

Pox

Papilloma

Polyomavirus

b- SS DNA include parvovirus B19

2- RNA Viruses

+SSRNA: Calcivirus, Picorna, Flavivirus, Togavirus, Coronavirus, Retrovirus.

-SSRNA: Paramyxovirus, Rhabdoviridae, Filovirus, Orthomyxovirus, Bunyavirus, Arenavirus.

Cellular response to viral infection

The cellular effect of virus infection includes cell shrinking, rounding, inclusion bodies, and the formation of giant multinucleate cells. This can result in cell death due to cytolysis or inhibition of cell metabolism.

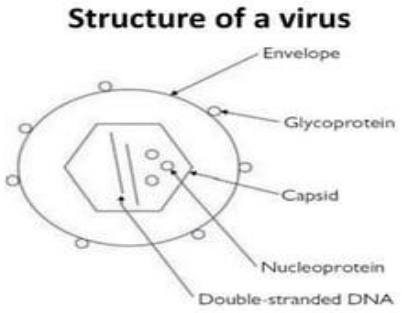
Chronic infection can result in:

- Latency: the viral genome is integrated into the host DNA. It does not replicate until a trigger occurs, e.g. stress. A classic example of this is herpes simplex virus.
- Persistence: the virus replicates in very low rate.
- Transformation: the virus initates new cell properties, which lead to teratogenic change.

Host response to viral infection

- Innate immunity: in the form of structural barriers (cilia in the respiratory tract, acid in the stomach) can prevent viruses invading the epithelial surface. The presence of fever inhibits viral replication.

- The humoral response: includes production of antibody, complement, and interferon and the cellular production of T and B lymphocytes, macrophages, and polymorpho-nuclear leukocytes.



Ocular Virology

Eye infection by viruses most often follows direct contact with virus externally, either from infected secretions in the birth canal (herpes simplex virus, human papillomavirus),
on fomites (adenovirus),
or airborne particles (rhinovirus),
or is acquired during viremia (human cytomegalovirus, measles virus). Other mechanisms of ocular viral infection include
-extension from contiguous adnexal disease (herpes simplex virus),
-spread from the upper respiratory tract via the nasolacrimal duct (rhinovirus),
-and transplacental passage of infectious virus (rubella virus). Rarely, ocular infection may disseminate elsewhere (enterovirus 70).

Acute viral infection produces stereotypic changes in ocular target tissues. Infection of the eyelid skin induces the formation of vesicles and ulcers. Viral infection of the conjunctiva results in vasodilatation, serous discharge, hyperplasia of conjunctival lymphoid follicles, and enlargement of the corresponding draining lymph nodes. Severe conjunctival infection can cause permanent scarring of the globe to the eyelids and turning in of the eyelashes against the eye. Viral infection of the corneal epithelium induces punctate epithelial cytopathic effect evident biomicroscopically as isolated swollen epithelial cells (punctate epithelial keratitis) and loss of individual epithelial cells (punctate epithelial erosions). When extensive, the punctate erosions may coalesce to form confluent epithelial ulcers with dendritic, dendritiform, or geographic morphology. With herpetic infection, corneal anesthesia can ensue, and in the absence of epitheliotropic neural growth factors, corneal epithelial integrity is impaired. Reduced corneal clarity and progressive sterile ulceration may result. Corneal stromal infection induces white blood cell recruitment; subsequent corneal scarring, vascularization, and lipid deposition may permanently reduce vision. Intraocular infection manifests in inflammatory cell deposits on the posterior surface of the cornea and on the vitreous scaffold, and in free-floating leukocytes and biomicroscopically visible protein spillage into the normally cell-free and protein-poor aqueous humor. Iridocorneal and iridolenticular adhesions may develop and lead to glaucoma and cataract. Retinal infection concludes with necrosis and lost function. Viral encephalitis and meningitis can result in cranial nerve inflammation and secondary dysfunction of vision and extraocular motility.

Classical viral pathogenic mechanisms of latency, reactivation, and carcinogenesis all can be demonstrated in the eye. Herpes simplex virus causes recurrent lytic epithelial keratitis when viral reactivation

within sensory ganglia of the first division of the fifth cranial nerve gives rise to virus in the preocular tear film. Necrotizing herpes stromal keratitis follows viral reactivation within the cornea stroma. Intraepithelial neoplasia and invasive squamous cell carcinoma of the conjunctiva and cornea have been associated with human papilloma virus types 16 and 18. When infected with oncogenic human papillomaviruses, corneal limbal stem cells can provide a persistent source of dysplastic ocular surface epithelium. Molecular mimicry has also been demonstrated as an immunopathogenic mechanism in ocular disease. Systemic infection with hepatitis C virus is associated with autoimmunity against a corneal stromal antigen and peripheral ulcerative keratitis. In a murine model of herpes simplex infection, non-necrotizing stromal keratitis accompanies T-cell reactivity against a corneal protein

Ocular Disease Caused by DNA Viruses

Table (1): DNA viruses are responsible for most significant ocular viral infections in the industrialized world. Even the protean ocular manifestations of the HIV, an RNA virus, result largely from reduced immunity to DNA viruses.

Virus	Family	Subfamily/genus	Nucleic acid	Env.	Ocular target
Adenovirus	Adenoviridae	Mastadenovirus	ds	–	Conjunctiva
					Cornea
Herpes simplex virus, type 1	Herpesviridae	Alphaherpesvirinae/Simplexvirus	ds	+	Eyelid
					Conjunctiva
					Cornea

Virus	Family	Subfamily/genus	Nucleic acid	Env.	Ocular target
(HHV1)					Trabecular meshwork
					Uvea
					Retina
Herpes simplex virus, type 2 (HHV2)	Herpesviridae	Alphaherpesvirinae/Simplexvirus	ds	+	Eyelid
					Conjunctiva
					Cornea
					Trabecular meshwork
					Uvea
					Retina
Varicella zoster virus (HHV3)	Herpesviridae	Alphaherpesvirinae/Varicellovirus	ds	+	Eyelid
					Conjunctiva
					Cornea
					Trabecular meshwork
					Uvea
					Retina
					Optic nerve
Epstein–Barr virus (HHV4)	Herpesviridae	Gamma herpesvirinae/Lymphocryptovirus	ds	+	Lacrimal gland
					Conjunctiva
					Cornea
					Uvea
					Retina

Virus	Family	Subfamily/genus	Nucleic acid	Env.	Ocular target
					Optic nerve
Human cytomegalovirus (HHV5)	*Herpesviridae*	*Betaherpesvirinae/Cytomegalovirus*	ds	+	Retina
					Optic nerve
Human herpes virus 6 (HHV6)	*Herpesviridae*	*Betaherpesvirinae/Roseolovirus*	ds	+	Retina
Human herpes virus 8 (HHV8)	*Herpesviridae*	*Gammaherpesvirinae*	ds	+	Conjunctiva (Kaposi sarcoma)
Human papillomavirus	*Papovaviridae*	*Papillomavirus*	ds	−	Eyelid
					Conjunctiva
					Cornea
Molluscum contagiosum virus	*Poxviridae*	*Molluscipoxvirus*	ds	+	Eyelid
					Conjunctiva
					Cornea
Orf virus	*Poxviridae*	*Parapoxvirus*	ds	+	Eyelid
Smallpox (variola) virus	*Poxviridae*	*Orthopoxvirus*	ds	+	Eyelid
					Conjunctiva
					Cornea
					Uvea
					Optic nerve
Vaccinia virus	*Poxviridae*	*Orthopoxvirus*	ds	+	Eyelid
					Conjunctiva

Virus	Family	Subfamily/genus	Nucleic acid	Env.	Ocular target
					Cornea

Ocular Virology includes:

1- Herpes
2- VZV (Varicella Zoster Virus)
3- HIV ocular infections
4- Pathological finding in the eyes for all the common viral infection

Important ocular viral pathogens

DNA viruses: herpes, adeno, papo, pox.

Herpes viruses: they are double strand DNA with an icosahedral capsid. An important feature of some of the herpes family, namely herpes simplex and varicella Zoster, is that they posses the ability to lie dormant in neuronal sites, and reactivate under certain conditions e.g. stress or other illness. This is known latency. Members of herpes family are the causative agents of acute retinal necrosis.

The herpes virus family includes:

1- Herpes simplex
2- Varicella zoster
3- Cytomegalovirus
4- Epstein-Barr virus (EBV).

Herpes simplex virus

Herpes simplex type 1 (HSV-1) is most commonly associated with oral infection (cold sores). Normally, the primary infection is in

childhood and is sub-clinical, with the cold sore occurring as a consequence of viral reactivation.

HSV-1 is relevant in ocular disease. It can give rise to conjunctivitis, keratitis, uveitis, and uveoretinitis.

Life cycle of HSV-1 include 4 stages:

1- Entry into the host and replication at the peripheral sites (mucosa, eye, skin)
2- Spread of the axonal terminals of sensory neurons followed by retrograde intra-axonal transport to neuronal cell bodies in sensory and autonornic ganglia
3- Latency ganglia, e.g. trigeminal ganglion.
4- Reactivation with the production in the ganglia of infectious virustransported anterogradedly to the periphery with further at the site of primary infection.

The pathogencity of herpes simplex is increased in immunosuppression, malignancy, and with the use of topical steroid

Reactivation itself can be triggered by these or hormonal changes, ultraviolet light, stress, and trauma.

The viral envelope is highly immunogenic and stromal disease, e.g. disciform keratitis, is due to hypersensitivity rection to viral antigen rather than the virus itself.

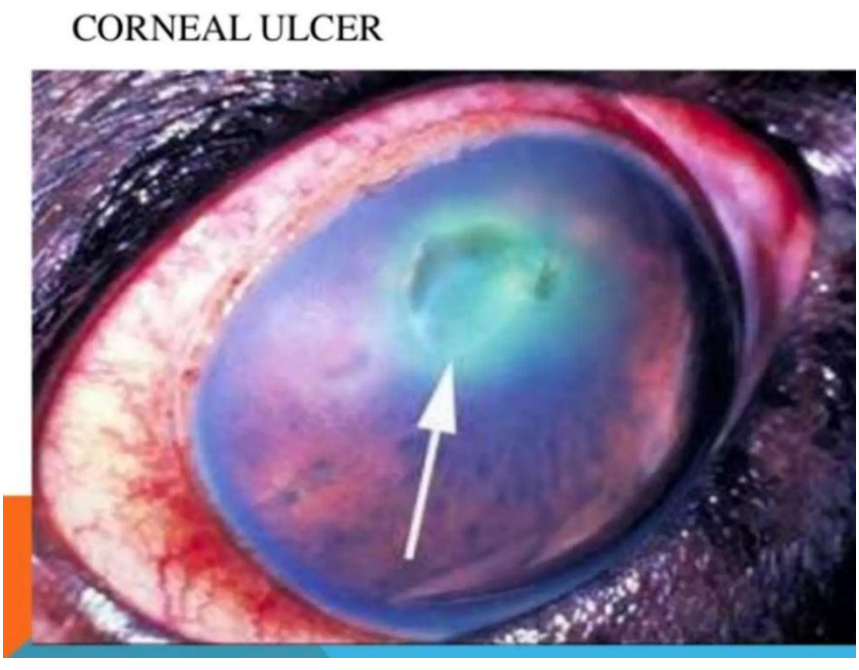
Clinical Features

- Epithelial dendritic, geographic keratitis causing ulcer which stains well with fluorescein dye.
- Ends of the ulcer has terminal buds staining with rose Bengal.
- Decreased corneal sensation
- Scarring and vascularization in keratitis, neurotrophic ulceration
- Keratoconjunctivitis and anterior uvetitis
- Preauricular lymphadenopathy, vesicular eruptions on the skin
- Fever and malaise

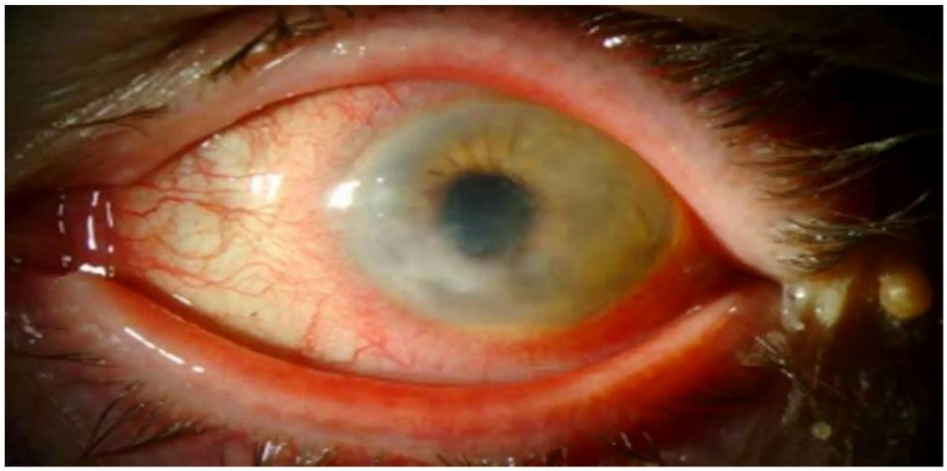
Diagnosis

Herpes zoster keratitis

Acanthaemoeba keratitis



Disciform keratitis in HSV



Herpes Simplex Keratitis

- Viral replication in cornea
- Symptoms: irritation, photophobia
- Signs:
  -red eye involving limbus
  -dendrite with terminal buds seen best with staining
  -ulcer formation

Varicella zoster virus (VZV)

Varicella zoster virus is the cause of chickenpox (collections of intraepidermal vesicles on the trunk, face, and mouth). Chickenpox is spread by respiratory route.

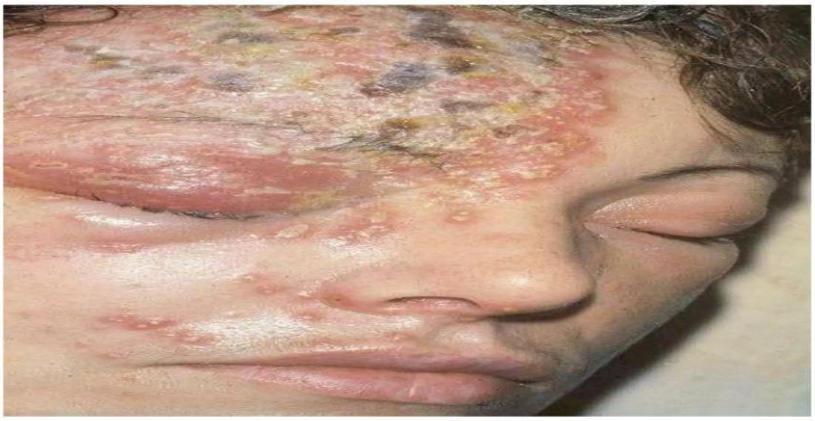
The virus can become latent in certain ganglia, most commonly the trigeminal, thoracic, lumbar, and cervical nerve ganglia.

Reactivation leads to shingles.

If reactivated in the trigeminal ganglion VZV can lead to ophthalmic shingles or herpes zoster ophthalmicus. Cell mediated immunity

maintains the virus in its latent state. Reactivation can be due to concurrent illness, immunosuppression or radiotherapy.

SHINGLES



Features

- Direct viral invasion – conjunctivitis- epithelial keratitis
- Secondary inflammation with episcleritis, scleritis, keratitis, uveitis, optic neuritis, cranial nerve palsy and cicatrizing complications of eyelids.
- Inflammation and destruction of the peripheral nerve or central ganglia may be responsible for post herpetic neuralgia
- Reactivation causes necrosis and inflammation in the sensory ganglia, corneal anaesthesia causing neurotrohic keratitis, skin vesicles occur which does not cross the midline

Treatment

- Oral- ointment- topical Acyclovir.
- Acyclovir cream for skin lesions
- Ganciclovir 0.15% gel
- Oral valaciclovir- famciclovir
- Interferon monotherapy

- Prostaglandins for IOP control
- Topical- oral steroids

Cytomegalovirus (CMV)

Cytomegalovirus infection is very common, but it is subclinical in 80% of cases. The virus is shed from the genital or urinary tracts and becomes latent in lymphocytes. Reactivation can occur during pregnancy leading to asymptomatic infection of the foetus. If the primary infection occurs in pregnancy, congenital anomalies can occur (this is more likely if primary infection is in the first trimester).

Congential infection with CMV can cause cytomegalic inclusion disease leading to

- Strabismus
- Chorioretinitis
- Microphthalmia
- Childhood hepatitis

Interestingly, the virus is shed at birth 1% of infants, with 10% of these having minor abnormalities, including hearing deficits

CMV infection in the immunosuppressed lead to:

- CMV retinitis (flashes, floaters, blurred vision, blind spots, specks invision)

- transplant rejection

- CMV pneumonia

Adenovirus

- Is a diverse group of double stranded DNA viruses consisting of over 30 antigenic types or serotypes.

- The infective particle has an icosahedral capsid without an envelope
- Transmission of the ocular infection is by direct contact with virus in ocular secretions. Contaminated instruments, eye drops or the hands of health professionals can be the fomite.
- Adenovirus is highly profilic: a single infected cell can produce 10.000 virions per cycle of 30-36 hours

Syndrome	Adenovirus type
Pharyngo conjunctival fever, ARD	3, 4, 7, 14, 21
Follicular conjunctivitis	1, 2, 3, 5, 6, 7
Epidemic keratoconjunctivitis (Sphipyard eye)	8, 19, 37

Signs

Conjunctiva- Eyelid edema, preauricular lymphadenopathy, conjunctival congestion, follicles, chemosis, Pseudo- membranes leaving a mild scar after resolution

Cornea: Non-staining epithelial microcycts, punctate epithelial keratitis, focal subepithelial /anterior stromal infiltrates. Mild anterior uveitis in some cases.

Lab diagnosis

1- Giemsa stain-mononuclear cells
2- Electron microscopy

3- Virus isolation from conjunctival swab
4- Cell line-primary human embryonic kidney cells, human epithelial cells and MRC-S cells (observe cytopathic effect) = plaques
5- Complement fixation test
6- ELISA & Enzyme immunoassays
7- PCR

Treatment

1- Topical antibiotics
2- Hygiene-hand wash
3- Lubricants
4- Topical steroids
5- Discontinue contact lens
6- Warm compressions

Papovarius

Human papillomavirus

Human papillomavirus is a double stranded DNA virus of which there are over 60 types.

HPV6 and HPV11 are associated with benign conjunctival papillomata

Malignant changes is caused by insertion of a particular type of HPV DNA into the host genome.

Pox virus

Pox viruses are double stranded DNA that replicate in the cytoplasm with very limited nuclear involvement. This is because they possess a DNA-dependent RNA-polymerase, a transcript poly-A polymerase, a

capping enzyme, and methylating enzymes, hence allowing them to replicate independently of the host cell.

Molluscum contagiosum

Molluscum contagiosum is the most common pox virus to cause ocular infection. It causes conjunctivitis flesh-coloured lesions on the face, extremites and trunk. It is commonly seen in children and is spread by direct contact or through contaminated fomites or water. Adult cases tend to be sexually transmitted. Epidermal hyperplasia is due to the production of a protein related the conserved domain of epidermal growth factor.

Molluscum contagiosum

RNA viruses; Retroviruses, Toga, Paramyxo

Human immunodeficiency virus (HIV)

HIV-1 and HIV-2 are retroviruses possessing the ability to infect CDA+ lymphocytes. They contain single strand RNA and reverse transcriptase. They differ in the structure of their glycoprotein envelopes.

Diagnostic tests for HIV:

Non- specific tests to detect HIV

- Leucocyte <2000/ mm^3
- Total CD4 count<200/ mm^3
- Reversal of CD4: CD8 ratio
- Raised IgG and IgA levels
- Decreased platelet counts- thrombocytopenia
- Diminished CMI by skin tests

Specific tests for HIV

HIV antibody: ELISA, Western blot, HIV differentiation assay.

HIV antigen detection: P24

HIV nucleic acids: PCR can be used to amplify the HIV genome RNA.

HIV culture: Virus isolated by co-culture with IL-2

Common ocular lesions in AIDs

Adenexa

- Conjunctival microvasculopathy
- Herpes zoster ophthalmicus
- Kaposi´s sarcoma of eyelid, conjunctiva
- Molluscum contagiosum of the eyelid

Anterior segment

- Dry eye
- Infective keratitis (Varicella zoster, Herpes simplex, Microsporidia)
- Anterior uvetitis
    - Rifabutin induced

- Spillover from Cytomegalovirus retinitis
- Herpes zoster ophthalmicus

Posterior segment

- HIV retinopathy
- Cytomegalovirus retinitis
- Toxoplasmic retinochoroiditis
- Pneumocystis carinii choroidopathy
- Acute retinal necrosis
- Herpes zoster retinopathy
- Progressive outer retinal necrosis
- Endogenous endophthalmitis

Orbit

- Burkitt´s lymphoma
- Orbital cellulitis (*Aspergillus*)

Neuroophthalmology

- Carnial nerve palsies
- Papillo edema
- Optic atrophy

Treatment options for viral ocular infections in HIV/AIDS patients

Infection	**Treatment**
Molluscum contagiosum	Topical phenol, trichloroacetic acid or liquid nitrogen Excision or curettage
Herpes zoster ophthalmicus	Initial: acyclovir 10 mg/kg Ivq 8 h Maintaince: acyclovir 500 mg po 3-5 times in day
Cytomegalovirus	IV, intravitreal, or oral ganciclovir

	IV or intravitreal foscarnet IV cidofovir

Paramyxovirus

Measles

Measles infection is characterized by pyrexia, cough, coryza and conjunctivitis.

Complications include secondary bacterial respiratory infections, encephalitis and rarely subacute sclerosing panecephalitis (SSPE), which can be associated with chorioretinitis and maculopathy.

Measles-RUBEOLA

Enters the host via respiratory route and spreads to the lymph nodes.

Incubation period= 1-3 weeks

Both humoral and cell mediates immunity take part in combating infection, has a lifelong immunity

Ocular manifestations:

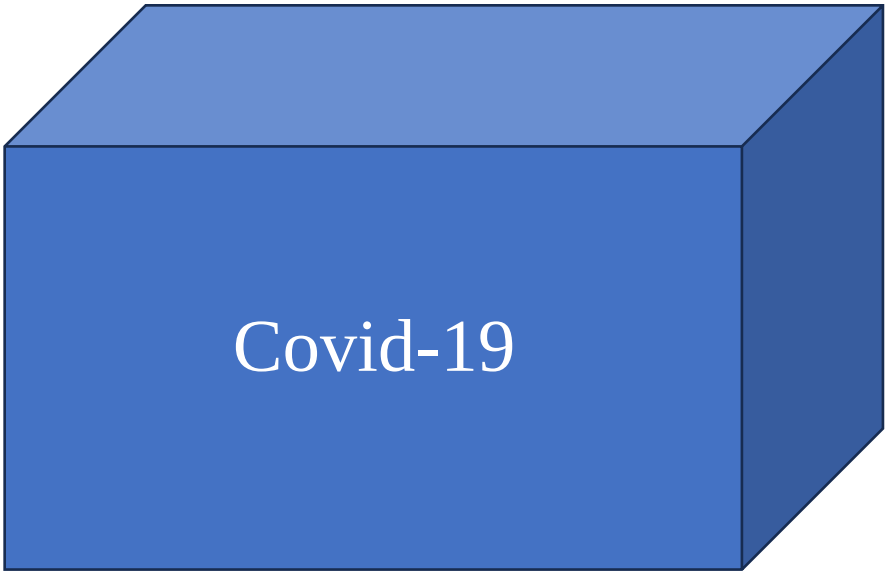
Conjunctivitis, Koplik´s spots

Toga virus

Rubella

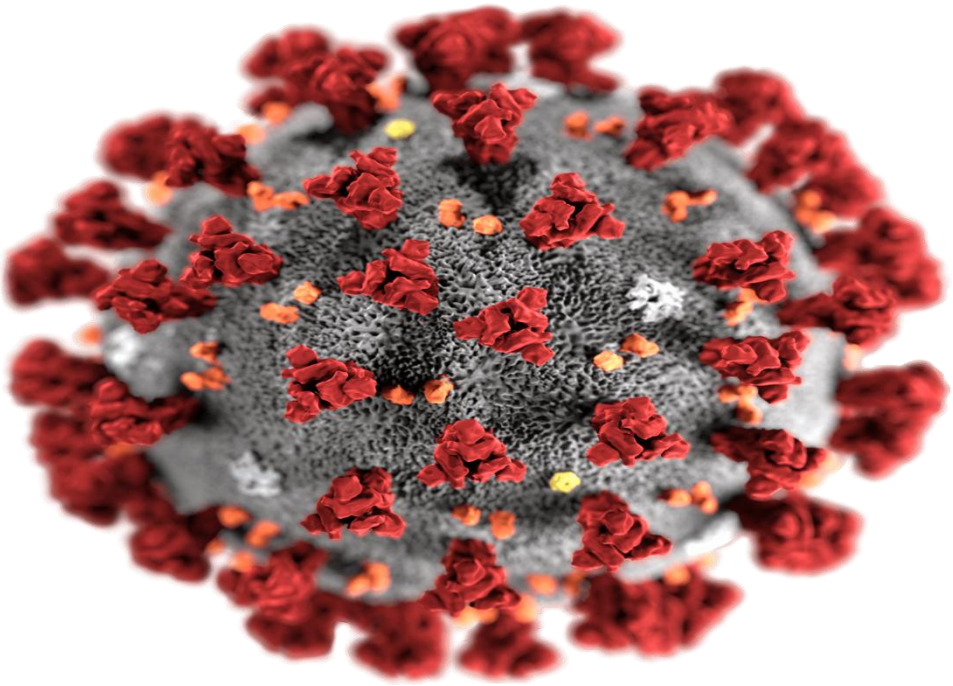
Rubella is subclinical in 80% of small children and 10% of adults. If a mother infected in the first trimester of pregnancy congenital anomalies occur due to spread to the placenta and hence foetus. Miscarriage or stillbirth may occur.

Congenital defects include cataract, microphthalmia, salt and pepper´retinitis, glaucoma, conductive deafness, and heart defects. For this reason, a live attenuated vaccine is given to children under the age of 12 years.

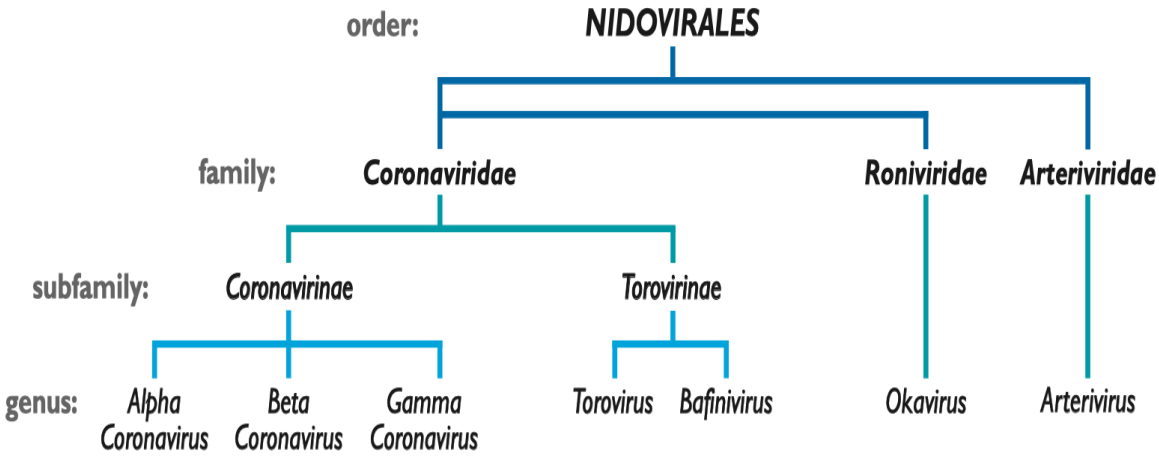


COVID-19

SARS-CoV-2



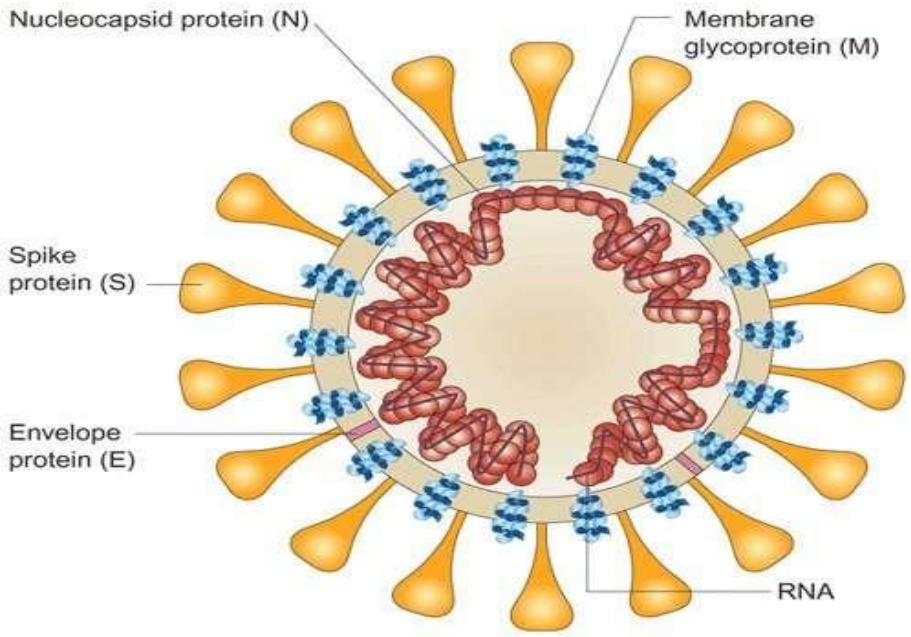
There are 7 Human Coronaviruses; 4 normal and 3 “novel”



Alpha: HCoV-229E, HCoV-NL63

Beta: HCoV-HKU1, HCoV-OC43, MERS-CoV, SARS-CoV, SARS-CoV-2

Coronavirus Structure



- Medium-sized virus size, but largest mRNA genome
- Enveloped +ve stranded RNA
- mRNA encased in nucleocapsid
- Lipid Bilayer – Soap works to disrupt this

Corona = Crowns for Spikes

- Glycoprotein Spike (S) Peptomer
- Spikes allow it to attach to human cell receptors in upper or lower airway

Coronavirus Genome

- Encodes four or five structural proteins:
    - **S** – spikes on the outside; **mediates receptor binding**
    - **M** – membrane protein; assists viral assembly

- **N** – nucleocapsid protein; regulation of viral RNA synthesis, may interact with M protein during virus budding
- **E** – small envelope protein; function necessary but not fully understood
- **HE** – hemagglutinin-esterase glycoprotein in Beta coronavirus OC43 and HKU1 only; enhances uptake into mucosal cells

Upper Respiratory Infections

URI viruses:

Rhinovirus

Influenza A/B

Adenovirus

Parainfluenza

Respiratory syncytial virus

Human metapneumovirus

- Normal human coronaviruses cause 5-10% of common cold/URIs, with outbreaks to 30% of common cold
  - **229E** and **NL63 (alpha coronaviruses)**
  - **OC43** and **HKU1 (beta coronaviruses)**
- These four predominately attach to receptors in UPPER airway (receptors: aminopeptidase N, dipeptidyl peptidase 4)
- Seasonality unpredictable (generally winter, but persists year round), different pattern in tropics than temperate regions

- URI symptoms, croupy or dry cough, rarely pneumonia (except sometimes NL63, but usually just causes croup); Mild diarrhea in infants

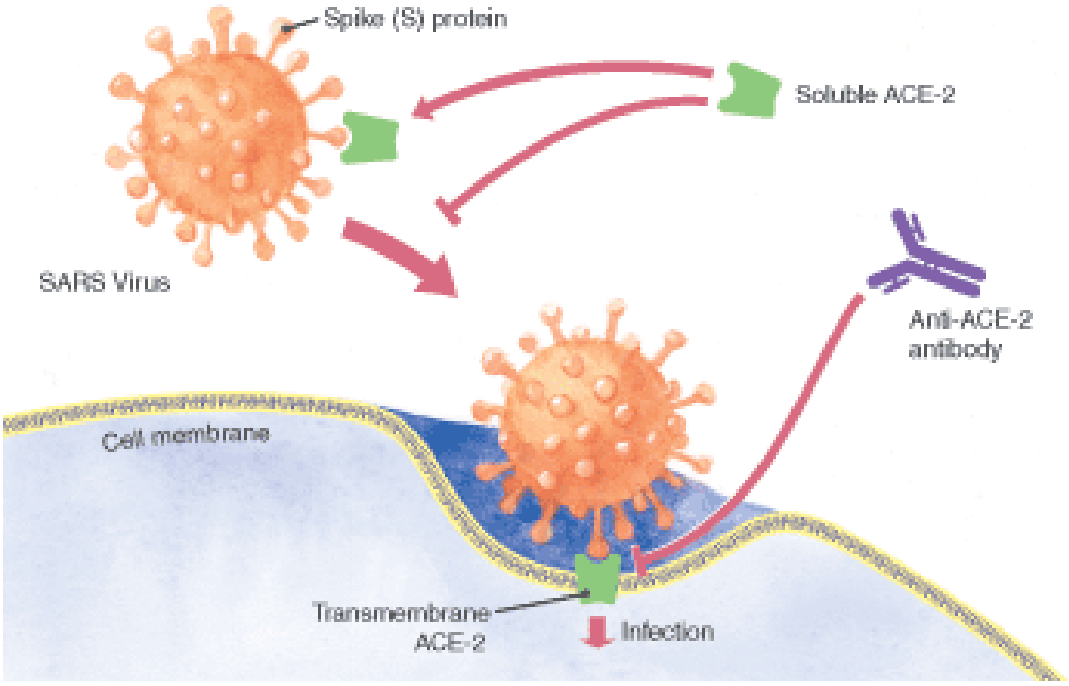
"Novel" Coronaviruses

- Novel coronaviruses predominantly in LOWER respiratory tract
    - **SARS, MERS, SARS-CoV-2**

Novel CoV attachment

- **ACE-2 Receptors**
    - Type 2 alveolar cells - highest
    - Bronchial epithelia
    - Tongue > buccal epithelia
    - Upper Intestinal epithelia
    - Myocardial cells
    - Kidney proximal tubule cells
    - Bladder urothelial cells
- SARS-CoV-2 binds to ACE-2 Receptor 10-20x more strongly than SARS-CoV
- Question of ADEs (Antibody Dependent Enhancement)
    - Antibodies can create a backdoor enhancement for viral replication

Implications on viral replication and vaccine development safety



SARS-Cov-2 origin

- Bat to a mammal (pangolin) to human in Nov/Dec 2019
- Pangolins used in Chinese medicine
- Probable link to seafood/exotic animal market
- Other plausible theory:
    - Wuhan Level 4 Biohazard lab experimental

animals sold for human consumption

SARS-Cov-2 Transmission

- Incubation 2-14 days (outlier 27 days)
    - Symptom onset median: Day 5-6 from exposure
- Doubling time: 6-7 days

- High viral shedding occurs early in disease course, even those with mild symptoms
- Prolonged shedding noted (unlikely reinfection)
- Up to 23% of transmissions due to pre-symptomatic cases in Shenzen
- Respiratory droplets (large - 3 ft, medium - 6 ft)
- Hand-to-mucus-membrane contact – sticks to skin easily!
    - T-zone: eyes, nose, mouth vulnerable
- Viable for 3 days on solids (plastics, porcelain, steel); ~24 hours cardboard, dependent also on temperature/humidity; 3 hours if aerosolized
- Airborne – likely not airborne with cough? But certainly, possible with intubation, non-invasive positive pressure ventilation, high flow O2, nebulizer, suctioning
- Fecal/oral – viral shedding present in stool and diarrhea is comm

Symptoms and Disease Course

- Week 1: Fever (77-98%) (intermittent or persistent), Fatigue/Malaise (11-52%), Dry cough (46-82%), dyspnea (3-31%);
    - Less common: Sputum (33%), Myalgia (15%), Headache (13%), Sore throat (14%), Diarrhea (4%), Nausea/Vomiting (5%), Nasal congestion (4%), Hemoptysis (1%)
- Week 2 (~ day 6-9 of symptoms): ~ 15-20% develop severe dyspnea due to viral pneumonia
    - Hospitalization, supportive care, oxygen

- Week 2-3: Of hospitalized patients, 1/3 ultimately need ICU care, with up to half needing intubation (i.e. ~5% of total diagnosed cases need ICU)
    - Can rapidly decline (over 12-24 hrs) from mild hypoxia to frank ARDS
    - Cytokine Storm, Multi-organ failure
    - Late-stage sudden cardiomyopathy/viral myocarditis, cardiac shock

Comorbidities and Risk Conditions

- Age
- HTN
- Diabetes
- Coronary Heart Disease
- Hep B
- Cerebrovascular Disease
- COPD
- Cancer
- Children and pregnant women seem to do okay

Ancillary Studies

- Most Common:
    - WBC usually normal, Lymphopenia in 80%, Mild thrombocytopenia
    - Low Procal; Bacterial coinfection rare

        - CRP and D-Dimer elevated proportionate to severity (marker of poor prognosis); DIC over time
        - Increased ALT/AST to 70-100 range; Occasional increased alk phos
        - Mild elevation of creatinine
        - Generally normal troponin
    - CXR (sensitivity 59%):
        - Bilateral patchy or reticular infiltrates, perihilar infiltrates occasionally
    - CT scan (sensitivity 86%; much better than RT-PCR!)
        - **Bilateral diffuse ground glass opacities**, **multifocal patchy consolidation**, interstitial changes
        - Changes prior to severe symptom onset!
    - ECHO:
        - Normal EF prior to late-onset sudden cardiogenic shock with dropping to EF <10%
        - Co-infection rare but possible (5%)

Person Under Investigation (PUI)

Clinicians should use their judgment. Most patients with COVID-19 have fever and/or cough or difficulty breathing.

Priority may be given to:

- **Hospitalized patients who have signs and symptoms** compatible with COVID-19 in order to inform decisions related to infection control precautions.

- **Symptomatic patients** such as, **older adults and individuals with chronic medical conditions** and/or an **immunocompromised state** (e.g., diabetes, heart disease, receiving immunosuppressive medications, chronic lung disease, chronic kidney disease).
- Any persons including **healthcare personnel**, who within 14 days of **symptom onset** had **close contact** with a suspect or laboratory-confirmed COVID-19 patient, or who have a history of travel from affected geographic areas within 14 days of their symptom onset.

Close contact is defined as—

a) being within approximately 6 feet (2 meters) of a COVID-19 case for a prolonged period of time; close contact can occur while caring for, living with, visiting, or sharing a healthcare waiting area or room with a COVID-19 case – *or* –

b) having direct contact with infectious secretions of a COVID-19 case (e.g., being coughed on)

If such contact occurs while not wearing recommended personal protective equipment or PPE (e.g., gowns, gloves, NIOSH-certified disposable N95 respirator, eye protection), **criteria for PUI consideration are met**.

Testing

- RT-PCR:
    - Real-time Polymerase Chain Reaction of RNA
        - Nasal AND Orophangeal Swabs (Collect 2 swabs)
        - Sputum better (but more dangerous to collect)
        - Stool – not generally used for testing

        - Blood or urine – virus not detected; blood could be tested for IgM, IgG later. DO get (bacterial) blood cultures for any sick patient.

- PCR ~ **60-80% sensitive**
    - A single negative RT-PCR *doesn't* exclude COVID-19 (*especially* if obtained from a nasopharyngeal source or relatively early in the disease course).
    - If RT-PCR is negative but suspicion remains, consider ongoing isolation and re-sampling several days later.
    - Sensitivity from private labs may vary; no data yet. Also dependent on collection technique and timing – early test on asymptomatic may not be accurate

Practical Part

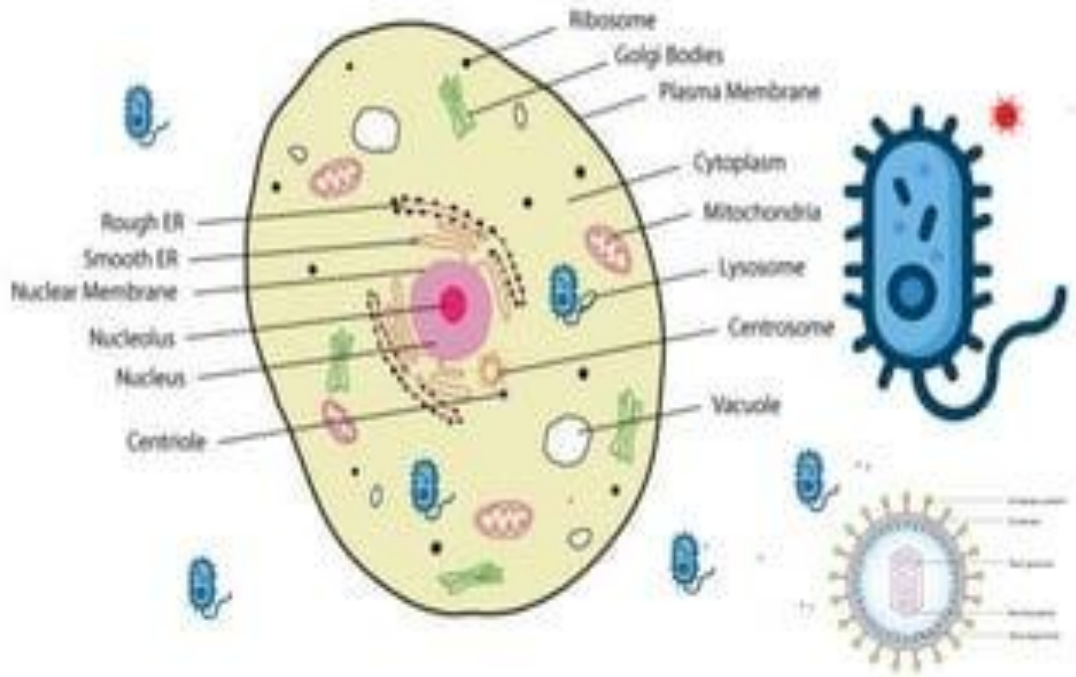
Viruses are the smallest infectious agents (20-300 nm) only seen under electron microscope (except poxviruses).

Electron microscope

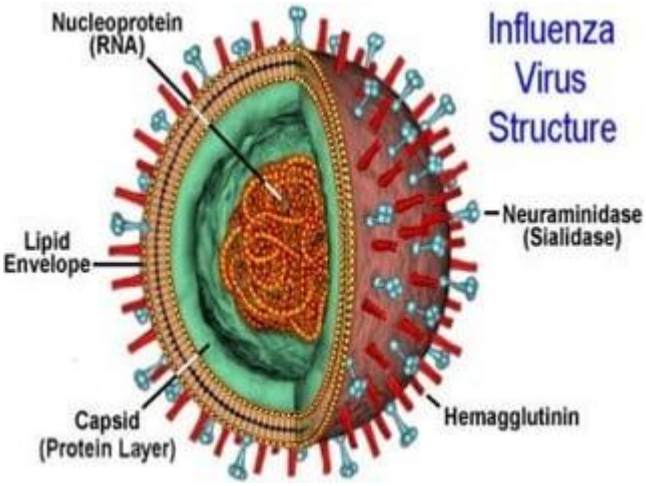
The source of illumination is a beam of electrons

Electromagnetic lenses

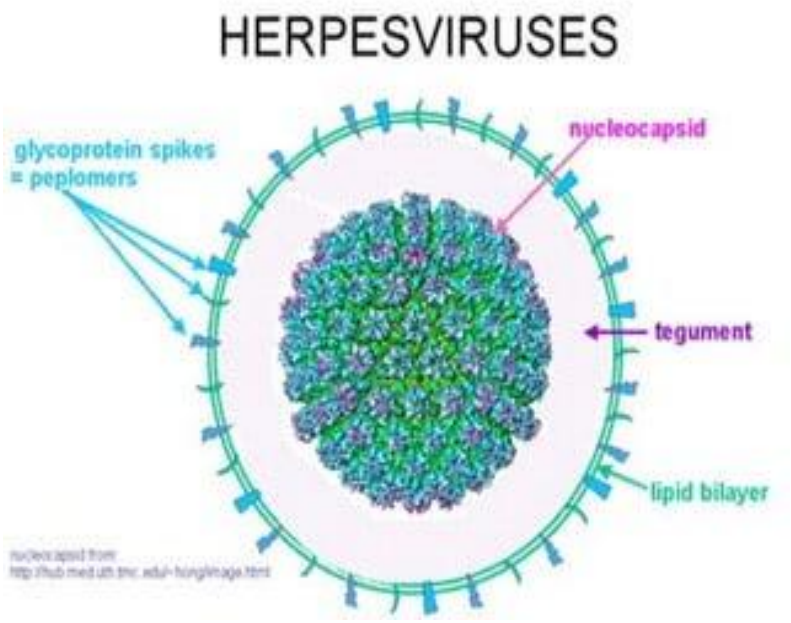
Magnification is 100.000 or more



Influenza virus



Herpes virus

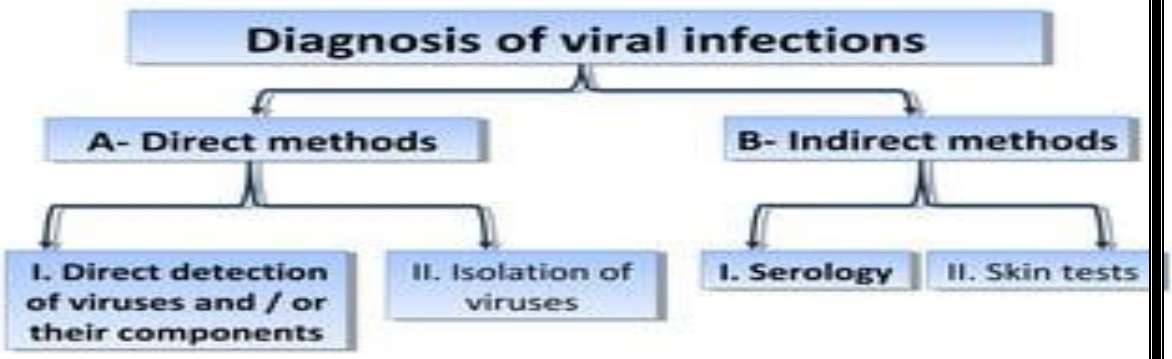


Differences between viruses and bacteria

1- They are obligatory intracellular parasites
    - They can not be cultivated or artifical culture media
    - Can only replicate inside living cells
2- They contain only one type of nucleic acid (RNA or DNA) not both

3- They are not suspectible to antibacterial agents

Diagonsis of viral infection

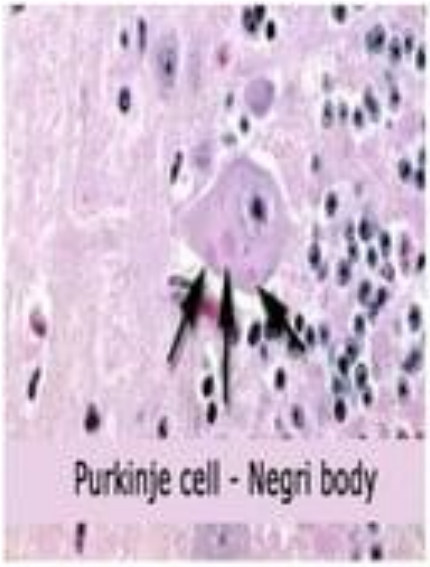


A- Direct methods

I. Direct detection of viruses & / or their components:

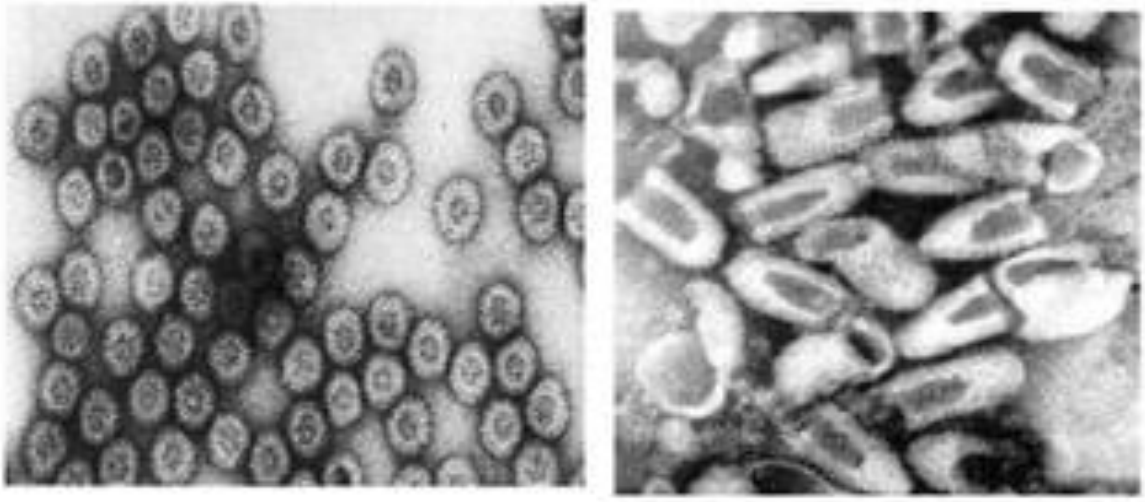
1. Light microscopy:

- Examination of large viruses as Poxviruses
- Detection of giant cells in Herpes infection
- Detection of inclusion bodies e.g. Negri bodies in nerve cells in rabies



2. Electron microscopy (EM):

- Large number of viruses in the sample.
- Size and shape of viruses.



3. Immunoelectron microscopy (IEM):

Sample (unknown virus) + known specific antibody

↓

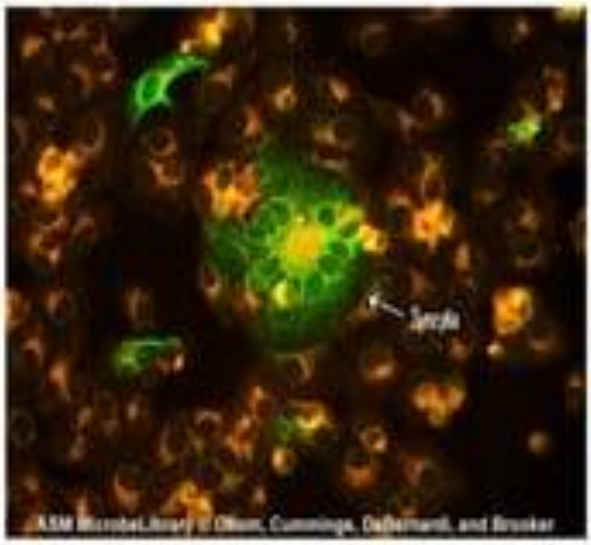
aggregation of unknown virus particles

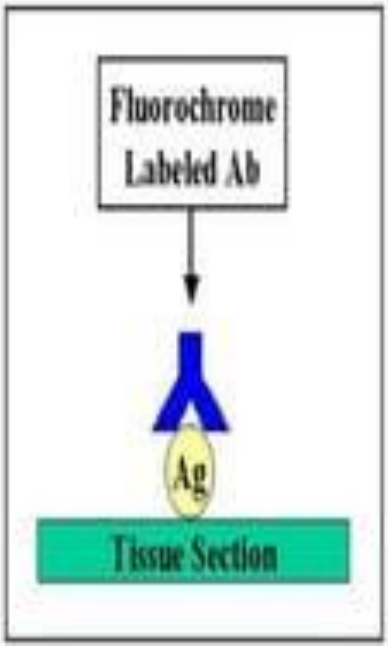
e.g. hepatitis A virus in stools

4. Fluorescent microscopy:

Direct immunofluorescent antibody technique (IF)

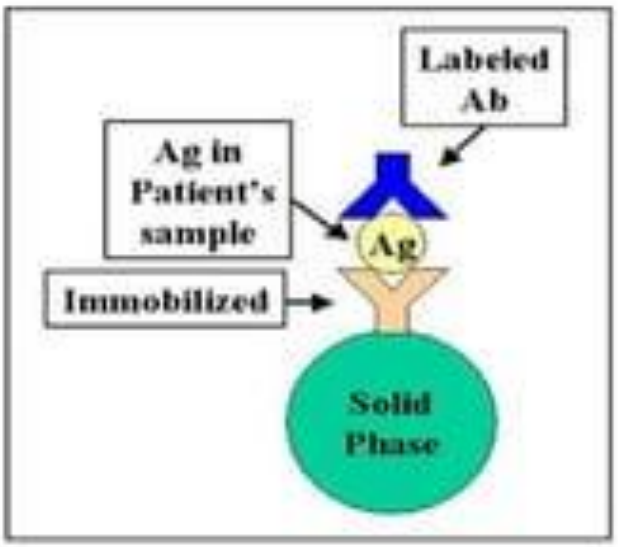
e.g. diagnosis of rabies in brain smears.

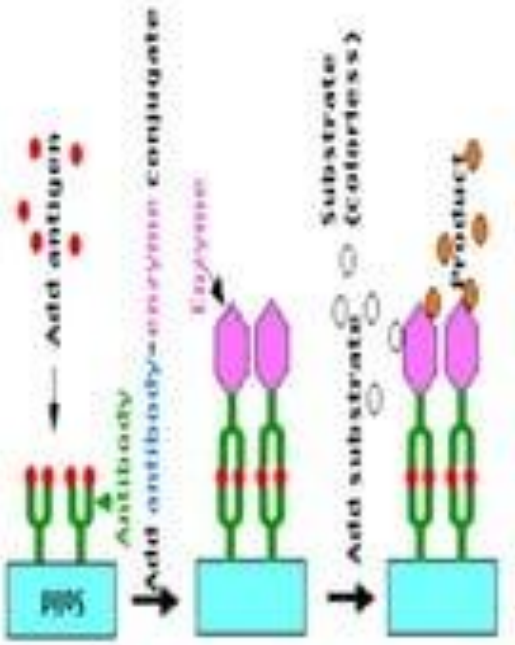




5. Immunoassays :

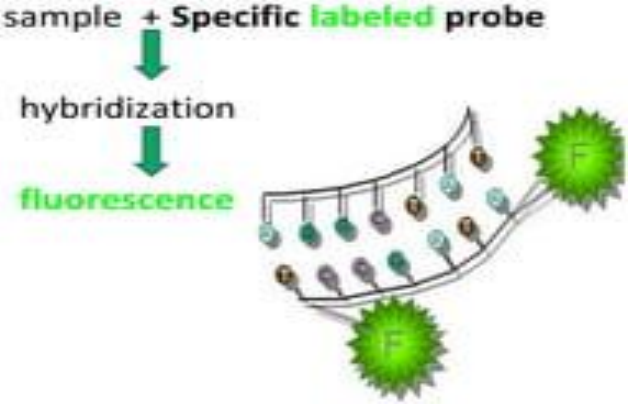
For detection of the virus antigens by ELISA / RIA
e.g. hepatitis B antigens in blood





6. Nucleic acid hybridization:

- A highly *sensitive* and *specific* method.
- Viral nucleic acid in sample + **Specific labeled probe**



7. Polymerase chain reaction (PCR)

Amplification of a specific sequence of nucleic acid

Detection e.g. by gel electrophoresis.

A- Direct methods

II. Isolation of viruses:

- Obligatory intracellular parasites
- Replicate only in living susceptible cells

a. Laboratory animals

b. Embryonated eggs

c. Cell culture (tissue culture)

Cultivation of viruses

a. Laboratory animals:

Used in:

- Isolation of coxsackie viruses by inoculation in **white suckling mice.**

- Rabies virus inoculation in **mice**.
- For research.

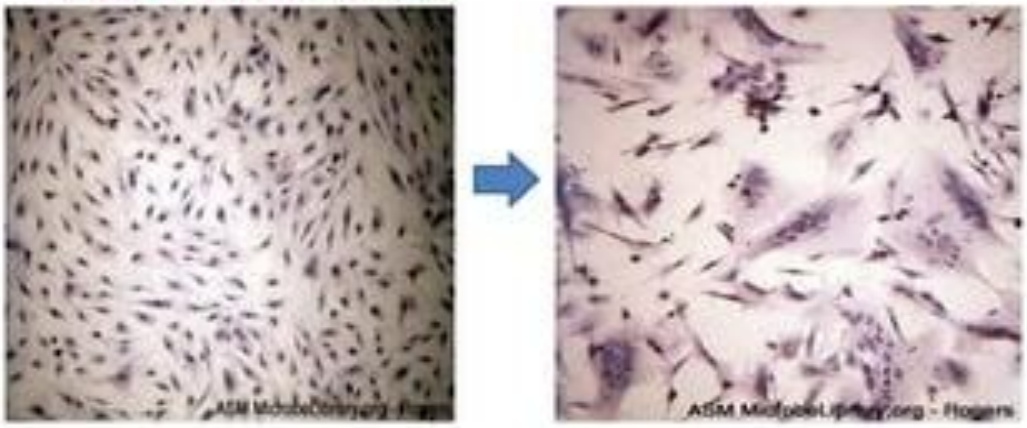
Types of cell lines

	Primary cell lines	Diploid cell lines	Continuous cell lines
Prepared from	Organ fragments	Human fibroblasts derived from embryonic tissues	Tumor cells
Examples	Monkey kidney	Human embryo lung tissue	HeLa cells from carcinoma of cervix
Number of Passages (subcultures)	5-10	50-100	Unlimited

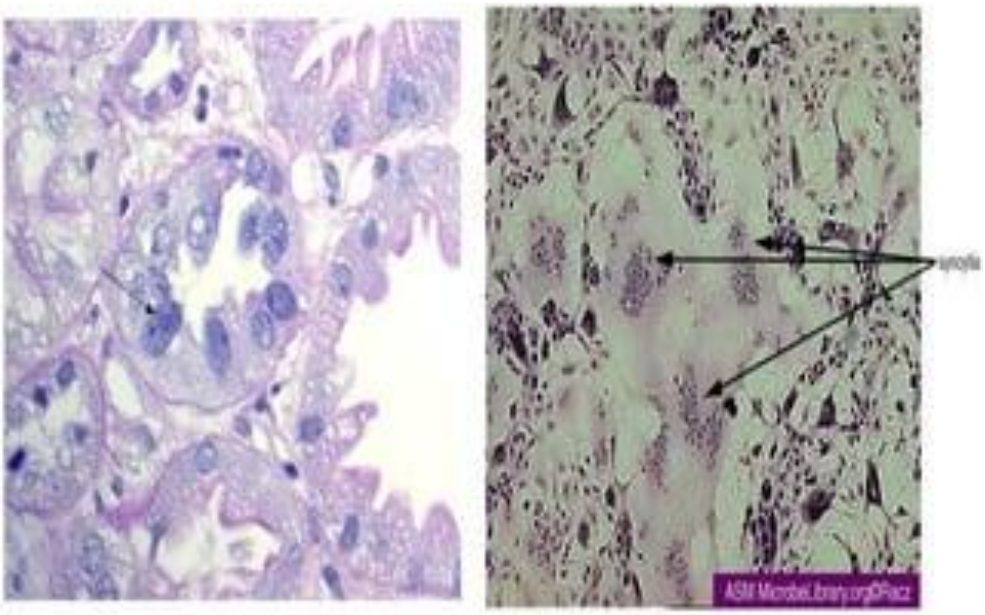
Detection of Virus Replication in Tissue Culture:

1- Cytopathic Effect (CPE):

i- Cell death or lysis

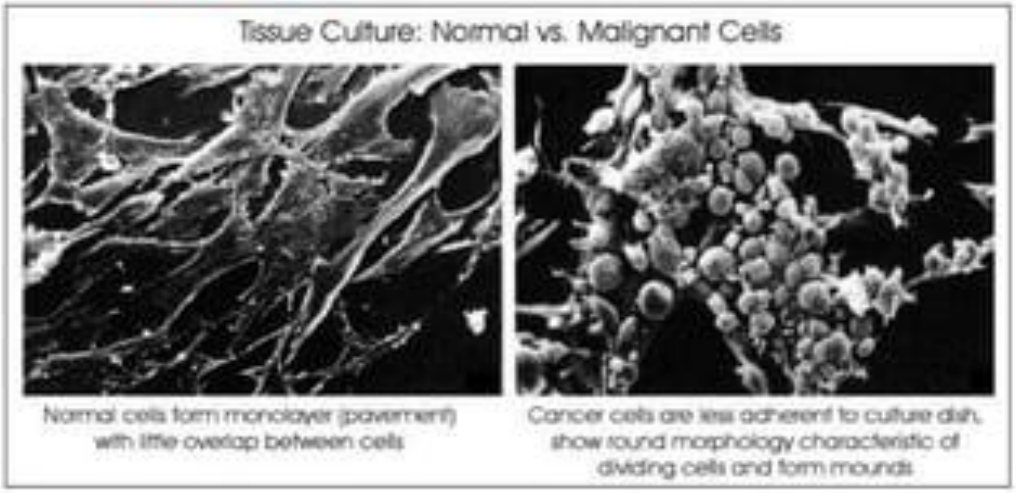


ii- Syncytial formation (multinucleated giant cells):



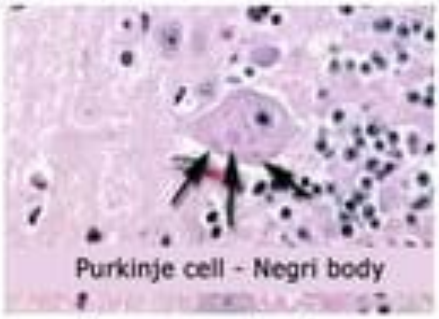
2- Transformation:

Viral nucleic acid + cellular DNA → cell transformation → foci of malignant cells.

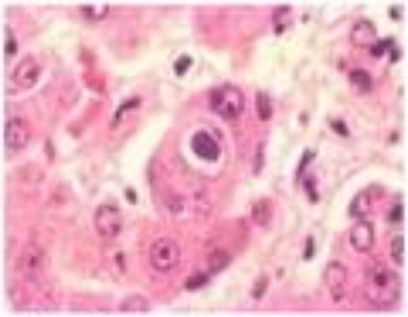


3- Inclusion bodies

- Site of **virus assembly** or **degenerative changes**
- Their location and appearance are **diagnostic** for a particular virus.



a- Intracytoplasmic: e.g. Rabies (Negri bodies)

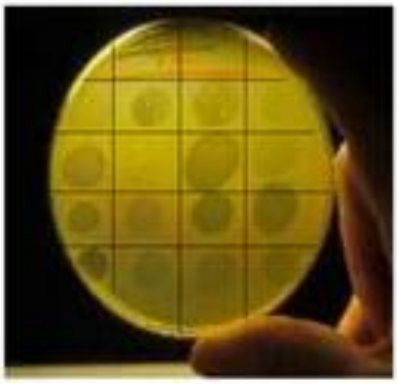


. b- Intranuclear: e.g Herpes viruses

c- Both: e.g. Measles virus and CMV

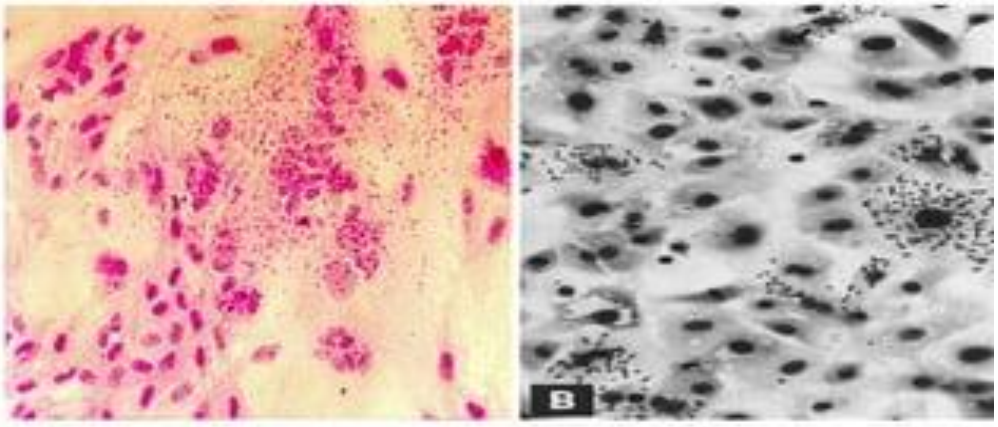
4- Plaque formation:

Infected monolayer + vital dye → unstained areas **(plaques)**



5- Haemadsorption:

Monolayer + hemagglutinating virus + RBCs →
hemadsorption (RBCs clumping) to infected cells



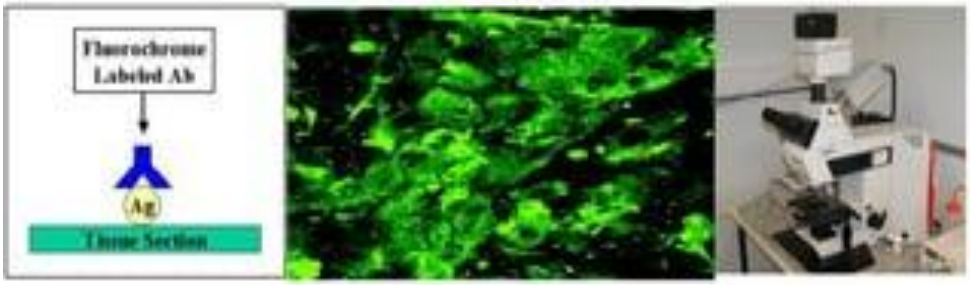
6. Interference phenomenon:

- Monolayer + rubella virus → no change for weeks
- Add CPE-producing virus → NO CPE

(due to interference)

7- Detection of viral antigens

8- Direct fluorescent antibody staining of infected cells (DFA):



:Neutralization test- 9

Monolayer + unknown virus + known specific Ab

NO CPE (due to neutralization)

B- Indirect methods

I. Serological diagnosis:

- Detection of **antiviral antibodies**

- 2 serum samples
  acute phase & 2-3 weeks later,
  to demonstrate a **rising titer**
  (4 fold increase or more is diagnostic).

- Only one sample may be used in the acute stage to detect **IgM**

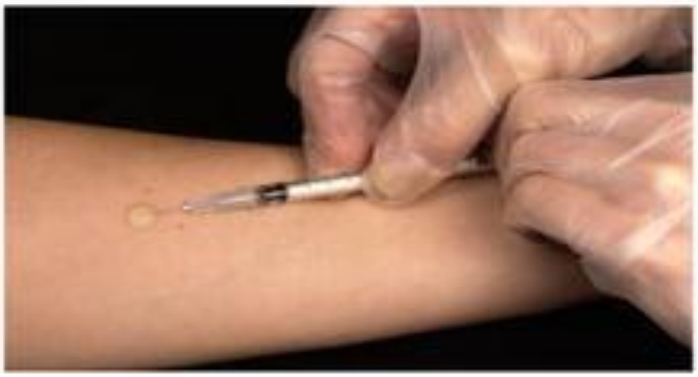
I. Serological diagnosis:

- Serological methods include:

- Neutralization test
- Complement fixation test
- Haemagglutination inhibition test
- Indirect IF
- ELISA
- RIA

II. Skin tests

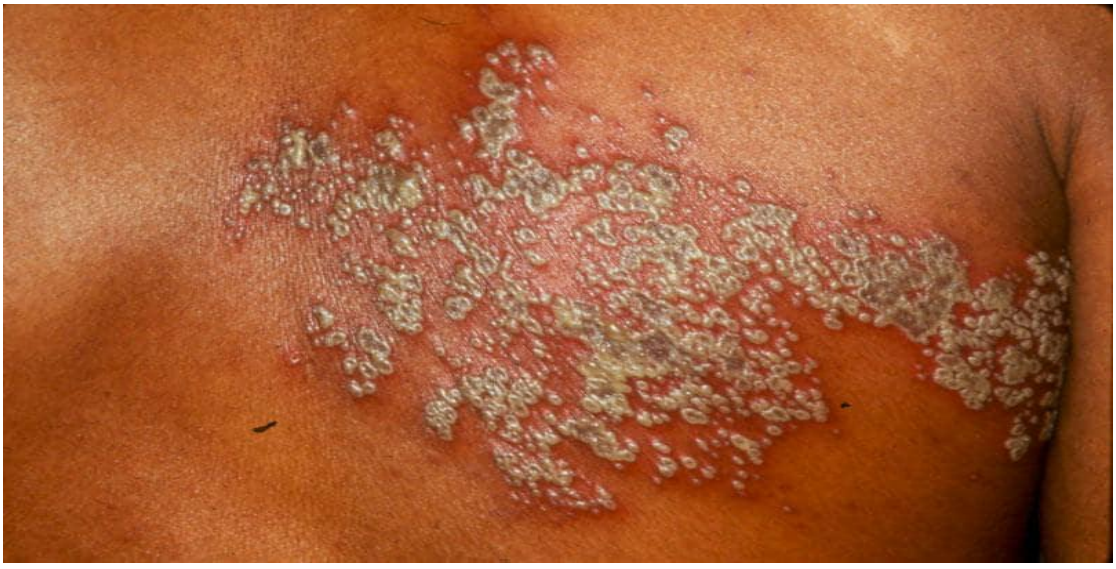
Used as an indication of cell-mediated immunity (CMI) in some viral infections, e.g. mumps.



Herpes Zoster infection

Herpes zoster is viral infection that occurs with reactivation of the varicella-zoster virus. It is usually a painful but self-limited dermatomal rash. Symptoms typically start with pain along the affected dermatome, which is followed in 2-3 days by a vesicular eruption. Classic physical findings include painful grouped herpetiform vesicles on an erythematous base. Treatment includes antiviral medications such as acyclovir, famciclovir, and valacyclovir given within 72 hours of symptom onset .

Reactivation of varicella-zoster virus (VZV) that has remained dormant within dorsal root ganglia, often for decades after the patient's initial exposure to the virus in the form of varicella (chickenpox), results in herpes zoster (shingles). While usually a self-limited rash with pain, it can be far more serious; in addition, acute cases often lead to postherpetic neuralgia (PHN) and is responsible for a significant economic burden. See the image below.



Signs and symptoms of herpes zoster

The clinical manifestations can be divided into the following three **phases:**

- Preeruptive phase (preherpetic neuralgia)
- Acute eruptive phase
- Chronic phase (PHN)

The preeruptive phase is characterized by the following:

- Sensory phenomena along 1 or more skin dermatomes, lasting 1-10 days (average, 48 hours)
- Phenomena usually are noted as pain or, less commonly, itching or paresthesias
- Pain may simulate headache, iritis, pleurisy, brachial neuritis, cardiac pain, appendicitis or other intra-abdominal disease, or sciatica
- Other symptoms, such as malaise, myalgia, headache, photophobia, and, uncommonly, fever

The acute eruptive phase is marked by the following:

- Patchy erythema, occasionally accompanied by induration, in the dermatomal area of involvement
- Regional lymphadenopathy, either at this stage or subsequently
- Grouped herpetiform vesicles developing on the erythematous base (the classic finding)
- Cutaneous findings that typically appear unilaterally, stopping abruptly at the midline of the limit of sensory coverage of the involved dermatome
- Vesicular involution: Vesicles initially are clear but eventually cloud, rupture, crust, and involute

- After vesicular involution, slow resolution of the remaining erythematous plaques, typically without visible sequelae
- Scarring can occur if deeper epidermal and dermal layers have been compromised by excoriation, secondary infection, or other complications
- Almost all adults experience pain, typically severe
- A few experience severe pain without a vesicular eruption (ie, zoster sine herpete)
- Symptoms tend to resolve over 10-15 days
- Complete healing of lesions may require up to a month

PHN is characterized by the following:

- Persistent or recurring pain lasting 30 or more days after the acute infection or after all lesions have crusted (9-45% of all cases)
- Pain usually is confined to the area of original dermatomal involvement
- The pain can be severe and incapacitating
- Pain can persist for weeks, months, or years
- Slow resolution of pain is especially common in the elderly
- PHN is observed more frequently after cases of herpes zoster ophthalmicus (HZO) and in instances of upper-body dermatomal involvement
- Less common postherpetic sequelae include hyperesthesia or, more rarely, hypoesthesia or anesthesia in the area of involvement

Common features of herpes zoster ophthalmicus are as follows:

- Classic symptoms and lesions of herpes zoster
- Ophthalmic manifestations including conjunctivitis, scleritis, episcleritis, keratitis iridocyclitis, Argyll-Robertson pupil, glaucoma, retinitis, choroiditis, optic neuritis, optic atrophy,

retrobulbar neuritis, exophthalmos, lid retraction, ptosis, and extraocular muscle palsies

Other forms include the following:

- Herpes zoster of maxillary branch of cranial nerve (CN) V
- Herpes zoster of mandibular branch of CN V
- Herpes zoster oticus (Ramsay Hunt syndrome)
- Glossopharyngeal and vagal herpes zoster
- Herpes occipitocollaris (vertebral nerves C2 and C3 involvement)
- Herpes zoster encephalomyelitis
- Disseminated herpes zoster
- Unilateral herpes zoster involving multiple dermatomes
- Recurrent herpes zoster
- Herpes zoster involving urinary bladder, bronchi, pleural spaces, or gastrointestinal tract
- Herpes zoster with motor complications

Diagnosis

Diagnosis is based primarily on the history and physical findings. In most cases, confirming the diagnosis via laboratory testing has no utility. In select patient populations, however—particularly immunocompromised patients—the presentation can be atypical and may require additional testing.

Laboratory studies for VZV include the following:

- Direct fluorescent antibody (DFA) testing of vesicular fluid or a corneal lesion
- Polymerase chain reaction (PCR) testing of vesicular fluid, a corneal lesion, or blood

- Tzanck smear of vesicular fluid (lower sensitivity and specificity than DFA or PCR)

Management

Episodes of herpes zoster are generally self-limited and resolve without intervention; they tend to be more benign and milder in children than in adults.

Conservative therapy includes the following:

- Nonsteroidal anti-inflammatory drugs (NSAIDs)
- Wet dressings with 5% aluminum acetate (Burrow solution), applied for 30-60 minutes 4-6 times daily
- Lotions (eg, calamine)

Primary medications for acute zoster–associated pain include the following:

- Narcotic and nonnarcotic analgesics (both systemic and topical)
- Neuroactive agents (eg, tricyclic antidepressants [TCAs])
- Anticonvulsant agents

Antibodies & Antigens

Antibodies are proteins that protect you when an unwanted substance enters your body. Produced by your immune system, antibodies bind to these unwanted substances in order to eliminate them from your system. Another word for antibody is immunoglobulin.

An antigen is a foreign substance that enters your body. This can include bacteria, viruses, fungi, allergens, venom and other various toxins. Antigen may be from the environment or from inside the body.

Where antibodies are produced?

Antibodies are produced by B cells (specialized white blood cells). When an antigen comes into contact with a B cell, it causes the B cell to divide and clone. These cloned B cells — or plasma cells — release millions of antibodies into your bloodstream and lymph system.

Antibodies are located in various areas of your body, including your skin, lungs, tears, saliva and even breast milk. In fact, high amounts of antibodies are present in colostrum (a thick fluid secreted by the breasts for a few days after giving birth). That's why breastfeeding (chest feeding) can boost your baby's immune system.

Monoclonal antibodies are created in a lab. They mimic your immune system's natural ability to fight off pathogens. Using monoclonal antibodies to fight infections is a type of immunotherapy.

Antibodies are categorized into five classes according to their location. Each one is labeled by a letter, which is attached to an abbreviation of the term "immunoglobulin" (Ig):

Antibody Type	Function

Antibody Type	Function
IgA	Found in saliva, tears, mucus, breast milk and intestinal fluid, IgA protects against ingested and inhaled pathogens.
IgD	This antibody is found on the surface of your B cells. Though its exact function is unclear, experts think that IgD supports B cell maturation and activation.
IgE	Found mainly in your skin, lungs and mucus membranes, IgE antibodies cause your mast cells (a type of white blood cell) to release histamine and other chemicals into your bloodstream. IgE antibodies can cause allergic reactions.
IgG	This is the most common antibody, making up approximately 70% to 75% of all immunoglobulins in your body. It's found mainly in blood and tissue fluids. IgG antibodies help protect your body from viral and bacterial infections.
IgM	Found in your blood and lymph system, IgM antibodies act as the first line of defense against infections. They also play a large role in immune regulation.

Antibodies are proteins. Each antibody has four polypeptides (peptides that consist of two or more amino acids), including two heavy chains and two light chains. Each antibody structure consists of two heavy chains and two light chains, which join to form a Y-shaped molecule. Each type of antibody has a different amino acid sequence at the tips of the "Y" which is why each antibody is shaped differently.

What conditions can monoclonal antibodies treat?

Each type of monoclonal antibody targets a specific antigen. As a result, monoclonal antibodies can treat a number of health conditions, including:

- Cancer.
- Rheumatoid arthritis.
- Heart disease.
- Multiple sclerosis (MS).
- Ulcerative colitis.
- Lupus.
- Crohn's disease.
- Psoriasis.
- Organ transplant rejection.

Covid antibodies

Antibodies to the virus that causes COVID-19 can be found in the blood of people who have recovered from the infection or those who have received the COVID-19 vaccine. If you've already had COVID-19, getting the vaccine increases your body's antibody response and improves your protection against the virus.

How long do COVID antibodies last?

Research is still ongoing, but studies show that people who had COVID-19 have antibodies for at least five to six months. One study found that people who've recovered from COVID-19 have memory B cells, which can stay in your body for years and target the virus specifically. This means you'll be

able to produce antibodies quickly if you're exposed to the virus again.

Thyroglobulin antibodies are often found in people who have thyroid problems, such as hyperthyroidism or hypothyroidism. These antibodies target thyroglobulin proteins (precursors of thyroid hormones) and can potentially destroy the thyroid gland.

Antithyroglobulin: If your healthcare provider suspects thyroid problems, they may recommend an antithyroglobulin antibody test. This test is also performed to monitor progress after thyroid cancer treatment.

If antithyroglobulin antibodies are found in your blood, then it could indicate thyroid problems, including:

- Hyperthyroidism.
- Hypothyroidism.
- Hashimoto's disease.
- Graves' disease.
- Subacute thyroiditis.
- Lupus.
- Type 1 diabetes.

Autoantibodies

Also known as antinuclear antibodies (ANAs), autoantibodies target normal proteins in a cell's nucleus. Autoantibodies mistake normal, healthy proteins as dangerous and unwanted. As a result, your body

begins attacking itself. Most people have small amounts of autoantibodies. In large quantities, however, autoantibodies usually indicate an autoimmune disease.

A blood test called the fluorescent antinuclear antibody test is the most common way to test for antinuclear antibodies. During this test, your healthcare provider views fluorescent-labeled antibodies under a microscope to determine the intensity and pattern of the fluorescence. This test is commonly used to rule out lupus.

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