MYCOLOGY AND VIROLOGY

Midterm

SYSTEMIC MYCOSES

The systemic diseases caused by fungi fall into two very distinct categories.

1. OPPORTUNISTIC FUNGUS INFECTION
2. TRUE PATHOGENIC FUNGUS INFECTIONS

Characteristics of true pathogenic fungi includes:

1. Restricted geographic distribution
2. Age, sex and race are important in the statistics of pathogenic fungus infection
3. Thermal dimorphism is exhibited by all of the major organisms that produce systemic infection

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TABLE 27-6 Summary of Systemic Mycoses

Fungus	Ecology	Clinical Disease	Tissue Form
Blastomyces dermatitidis	Mississippi and Ohio River valleys	Primary lung Chronic skin-bone Systemic, multiorgan	Large yeast (8-12 µm) Broad-based bud
*Histoplasma capsulatum**	Ohio, Missouri, and Mississippi River valleys Bird and bat guano Alkaline soil	Primary lung Asymptomatic Immunodeficient hosts prone to disseminated disease	Small, oval yeast (2-5 µm) in histiocytes, phagocytes
Coccidioides immitis, C. posadasii	Semiarid regions— southwest United States, Mexico, Central and South America In soil	Primary lung Asymptomatic Secondary cavitary Progressive pulmonary Multisystem	Spherules (30-60 µm) containing endospores
Paracoccidioides brasiliensis	Central and South America In soil	Primary lung Granulomatous Ulcerative nasal and buccal lesions Lymph node involvement Adrenals	Thick-walled yeasts (15-30 µm) Multiple buds, "Mariner's wheel"

*Histoplasma capsulatum var. capsulatum (teleomorph *Ajellomyces capsulatus*). *Histoplasma capsulatum var. duboisii* is endemic in Central Africa.

TABLE 27-4 Morphology of Systemic Fungi*

Fungus	Macroscopic Morphology	Microscopic Morphology
Blastomyces dermatitidis	Slow to moderate growth White to dark tan Young colonies tenacious, older colonies glabrous to woolly	Oval, pyriform, to globose smooth conidia borne on short, lateral hypha-like conidiophores
Histoplasma capsulatum	Slow growth White to dark tan with age Woolly, cottony, or granular	Microconidia small, one-celled, round, smooth (2-5 µm) Tuberculated macroconidia large, round (7-12 µm) Hypha-like conidiophores
Coccidioides immitis, Coccidioides posadasii	Rapid growth White to tan to dark gray Young colonies tenacious, older colonies cottony Tend to grow in concentric rings	Alternating one-celled, "barrel-shaped" arthroconidia with disjunctor cells
Paracoccidioides brasiliensis	Slow growth White to beige Colony glabrous, leathery, flat to wrinkled, folded or velvety	Colonies frequently only produce sterile hyphae. Fresh isolates may produce conidia similar to those of *B. dermatitidis*.

*At 22°C.

The TRUE PATHOGENIC FUNGI includes:

1. Histoplasma capsulatum
2. Blastomyces dermatidis
3. Coccidioides immitis
   Paracoccidioides brasiliensis
4. Penicillium marneffei

The OPPORTUNISTIC FUNGUS INFECTIONS

The organisms involved are inherently low virulence, and disease production depends on diminished host resistance to infection.

The etiologic agents of opportunistic infections are:

1. ASPERGILLUS,
2. CANDIDA,
3. CRYPTOCOCCUS
4. RHIZOPUS

CANDIDIASIS

- A primary or secondary infection caused by a member of the genus Candida
- **Candida albicans** > most prevalent specie in mucocutaneous and disseminated infections
- All of the tissue and organ systems are subject to invasion and the pathology evoked is as variable as are the clinical syndromes.

1. Infectious diseases

A. Mucocutaneous involvement:

- Oral thrush, glossitis, stomatitis, cheilitis (Fig. 4.1)
- Bronchial and pulmonary
- Vaginitis, balanitis
- Esophagitis, enteric and perianal disease

- Chronic mucocutaneous candidiasis (Fig. 4.2)

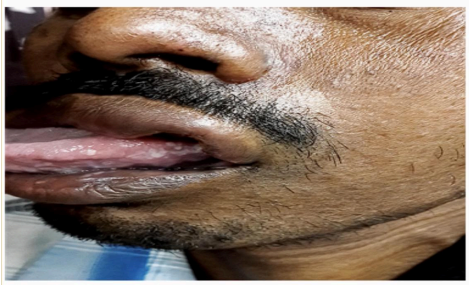


Fig. 4.1: Oral candidiasis—'thrush'. Cream white, cardy patches of pseudomembrane cover the front and lateral portion of the tongue in HIV reactive patient.



Fig. 4.2: Mucocutaneous candidiasis in HIV reactive patient.

B. Cutaneous involvement:

- Intertriginous and generalized candidiasis
- Paronychia and onychomycosis
- Diaper rash (napkin candidiasis)
- Candidal granuloma

C. Systemic involvement:

- tract
- Endocarditis
- Meningitis
- Septicemia
- Iatrogenic candidemia

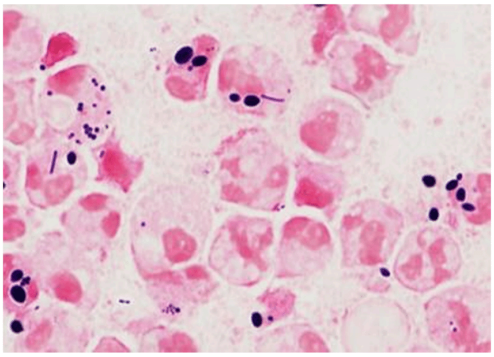
2. Allergic disease

- Eczema
- Asthma
- Gastritis,

Laboratory Identification

Direct examination

- Scrapings from cutaneous and mucocutaneous lesions can be examined directly either in KOH or by Gram stain
- finding of yeast in such material is of no diagnostic importance; however, mycelial form organisms usually connote an established colonization of the involved area.



Direct culture

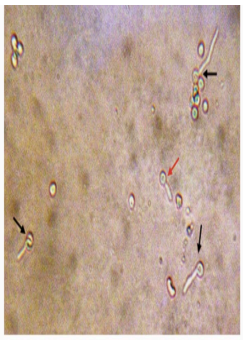
- Freshly obtained, multiple specimens should be examined for maximum isolation of fungi.
- **Sabouraud's dextrose agar** with antibacterial antibiotics is recommended.
- Optimal growth of all species occurs at room temperature.
- A pasty, yeast-like colony appears by 24 to 48 hours.



Fig. 4.4: Colony morphology on Sabouraud's dextrose agar; (a) *Candida albicans;* (b) *Candida tropicalis.*

Proposed rapid identification of unknown yeasts

1. Germ tube production (Reynolds-Braude phenomenon)
2. Chlamydospore production in Cornmeal Tween agar
3. Microscopic morphology (mycelial morphology) on Cornmeal-Tween plate
4. Carbohydrate fermentation
5. Carbohydrate assimilation

Reynolds-Braude Phenomenon **Unknown yeast** is mixed with serum and incubated at 37°C for two hours. C. albicans and C. dubliniensis shows sprout mycelium which are called 'germ tube'. **POSITIVE** if 30% yeast cells produce sprout mycelium.	 Fig. 4.3: 'Germ tube' formation (black arrow) in *Candida albicans* (Reynolds-Braude phenomenon) which differs from pseudohyphae formation (red arrow) [×400].
Microscopic Morphology on Cornmeal-Tween Agar • **Candida albicans** is the most common species causing human infection • White to cream colored, smooth and glistening (three days) • Cream colored, waxy or soft, smooth to reticulated or wrinkled with or without mycelial fringes.	 Fig. 4.4: Colony morphology on Sabouraud's dextrose agar; (a) *Candida albicans;* (b) *Candida tropicalis.*

Microscopic morphology

On SDA at 25°–30°C for three days, the colony is composed of globose to oval to elongate yeast cells of various sizes

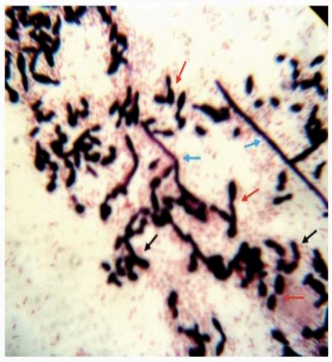


Fig. 4.5: Gram stained smear prepared from colonies appeared on Sabouraud's dextrose agar (SDA) at 37°C for 3 days. Yeast cells are oval to elliptical with budding (red arrow), a few cylindrical cells (black arrow) and also mycelial fragments (blue arrow) are noticed. *Candida tropicalis* [Urine sample of a 68 years old male patient with symptoms of UTI; ×1000].

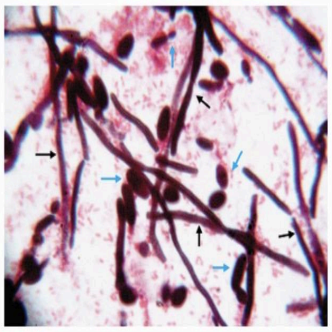


Fig. 4.7: *Candida albicans.* Oval to elongate budding yeast cells (blue arrow) with plenty of pseudomycelia (black arrow) are noticed in this smear prepared from colonies appeared on Sabouraud's dextrose agar following an inoculation of sputum sample at 37°C for 3 days. The sputum sample was obtained from a 21 years male patient of 'acute myeloblastic leukaemia' [Gram stain ×1000].

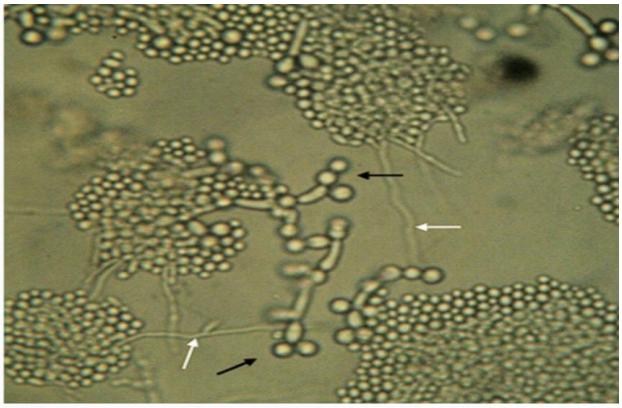


Fig. 4.11: *Candida albicans*. Large, thick-walle round resting spores borne terminally. Chlamyd spores are not produced at 30–35°C (×600).

Candida tropicalis

- **Colony morphology -** Colonies grown on SDCA at 25°C for 3 days are white to cream coloured, glistening to dull, soft, smooth, reticulated or wrinkled often with overgrowth of mycelium (Fig. 4.4). Old cultures become hairy and tough
- **Microscopic morphology -** The cells grown on Sabouraud's agar (SDCA) are globose, ovoid to elongated, measuring 4 to 7 × 5 to 11 µm (Figs 4.13 and 4.14). Pseudohyphae are long and branched, bearing blastospores singly or in small chains at the septa.

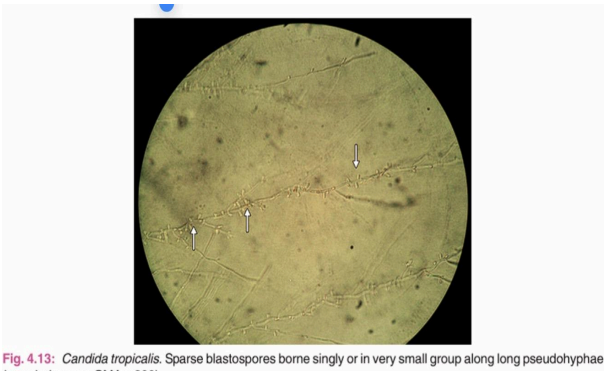


Fig. 4.13: *Candida tropicalis*. Sparse blastospores borne singly or in very small group along long pseudohyphae (morphology on CMA ×200).

Candida kefyr

Colony morphology

Colonies grown on Sabouraud's dextrose agar are cream coloured, semi-dull to glistening and indistinguishable from other species of Candida.

Microscopic morphology

The yeast cells are long ovoid, blastospores are scanty, pseudomycelia lie parallel to each other like logs in stream.

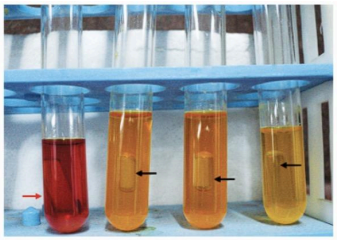


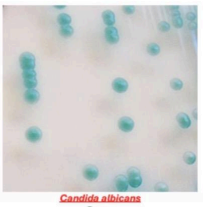
Fig. 4.20: *Candida tropicalis*. Glucose, sucrose and maltose are fermented with production of acid and gas (black arrow). Lactose is not fermented (red arrow).

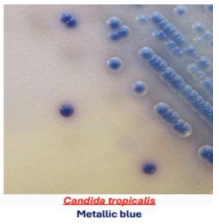


Fig. 4.22: *Candida kefyr*. Fragile pseudohyphae of long cells. These fall apart and lie parallel like "logs in a stream" (black arrow; ×400).



Fig. 4.15: Colony morphology on Sabouraud's dextrose agar; (a) *Candida parapsilosis*; (b) *Candida guilliermondii*.





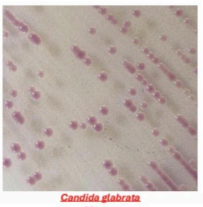






Fig. 4.19: Sugar fermentation by *Candida* species (*C. guilliermondii*). G—glucose, S—sucrose, M—maltose, L—lactose. Fermentation is indicated by production of acid and gas. Accumulation of gas at the top of the Durham's tube (black arrow) is more important feature than mere acid production.



Fig. 4.21: *Candida guilliermondii*. Colonies grown on SDCA at 37°C after 3 days of incubation.

	Temperature Growth at (°C) a	
	37°	42°
Candida		
C. albicans	+	+
C. dubliniensis	+	-
C. glabrata	+	+
C. guilliermondii	+	+
C. krusei	+	+
C. lusitaniae	+	+
C. parapsilosis	+	-
C. stellatoidea	+	+
C. tropicalis	+	+
Cryptococcus		
C. albidus	-	-
C. neoformans	+	-
Trichosporon spp.	+	V

	Cornmeal Agar		
	Pseudo Hyphae	True Hyphae	Arthroconidia
Candida			
C. albicans	+	+	-
C. dubliniensis	+	+	-
C. glabrata	-	-	-
C. guilliermondii	+	-	-
C. krusei	+	-	-
C. lusitaniae	+	-	-
C. parapsilosis	+	-	-
C. stellatoidea	+	+	-
C. tropicalis	+	-	-
Cryptococcus			
C. albidus	-	-	-
C. neoformans	-	-	-
Trichosporon spp.	+	+	+

	Susceptibility to* Cyclohexamide	Urea	Nitrate
Candida			
C. albicans	R	-	-
C. dubliniensis	R	-	-
C. glabrata	S	-	-
C. guilliermondii	R	-	-
C. krusei	S	V	-
C. lusitaniae	V	-	-
C. parapsilosis	S	-	-
C. stellatoidea	S	-	-
C. tropicalis	V	-	-
Cryptococcus			
C. albidus	S	+	+
C. neoformans	S	+	-
Trichosporon spp.	R	+	-

ASPERGILLOSIS

(2ND most isolated fungus next to candida, Commonly an opportunistic infection of bone marrow transplant recipients)

- **Aspergillus fumigatus –** most common cause of invasive and non-invasive aspergillosis
- **Aspergillus flavus –** the second most common specie causing invasive aspergillosis
- **Aspergillus niger –** the third most common cause of invasive pulmonary aspergillosis

Aspergillus flavus (aflatoxin) and Aspergillus parasiticus

is notorious in causing mycotoxicosis

Characteristics:

- Recovered in air-settling plates
- **Macroscopic examination:** o black to white and include yellow, brown, green, gray, pink, beige, and tan (depends on the conidia)

Microscopic examination

o parallel columns or in a radiating pattern of conidia



Clinical entities caused by Aspergillus species:

1. Allergy to Aspergillus antigen

- Allergic bronchopulmonary aspergillosis
- Sporophytic colonization of air spaces (otomycosis, fungal ball of paranasal sinuses, fungal ball of lung, endobronchial colonization).

2. Invasive aspergillosis.

- **Nonpulmonary portal of entry**

a. Eye—keratomycocis, exogenous endophthalmitis
b. Paranasal sinus, cardiac or vascular surgery, intravenous drug addiction, skin

- **Pulmonary portal of entry**

a. Lung abscess, pneumonia.

Laboratory Diagnosis

Direct examination

- **Aspergillus hyphae** in clinical specimens of sputum, sinus drainage, or bronchial washings can be readily detected by direct microscopic examination. Purulent or blood tinged, thick sputum may be digested with 10% potassium hydroxide before examination
- Long strands of hyphae with dichotomous branching and regular septation may be present. Occasionally whole conidial structures may be seen which indicates that Aspergillus is colonizing an area in contact with air

Isolation in culture

- Sabouraud's dextrose agar with antibiotic chloramphenicol (SDCA), cyclo hexamide should not be used as majority of the species of Aspergillus are sensitive to it.

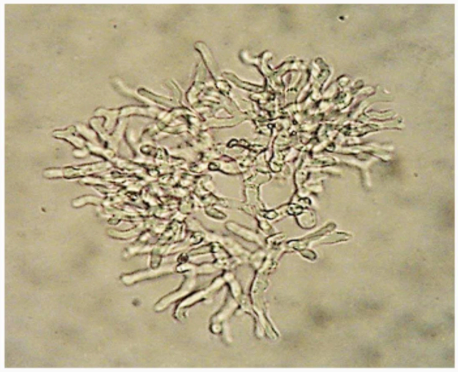


Fig. 4.42: Bronchopulmonary aspergillosis. Direct microscopy of sputum sample after digestion with 10% KOH (potassium hydroxide). Clumps of aspergillus are seen that demonstrate finger-like dichotomous branching [×400].

Aspergillus fumigatus

Colony morphology

The colonies have a velvety texture and spread broadly over the surface of Sabouraud's dextrose agar. The colour for the colony for the first 2 to 3 days is white, gradually turns into greenish blue to grayish green as the conidial heads mature (Fig. 4.43).

Reverse is usually colourless. The conidia masses of the conidial heads are columnar, compact and often crowded.

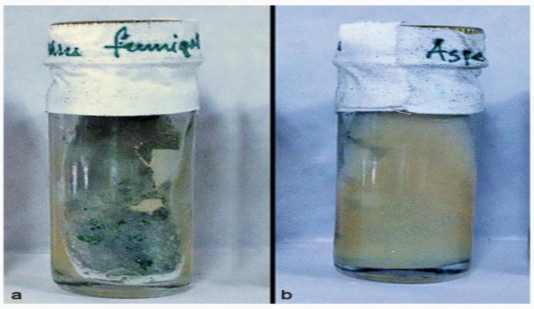


Fig. 4.43: Colony morphology of *Aspergillus fumigatus* grown on Sabouraud's dextrose agar at 25°C for 1 week; (a) obverse—flat, white for the first 2 to 3 days but soon become velvety in texture, greenish blue to grayish green with maturation of conidial heads and production of conidia, (b) reverse is colourless.

Microscopic morphology

Conidiophore is short, smooth and has a slightly green or brownish colouration especially towards the upper part near the vesicle. The conidiophores enlarge to form the flask-shaped vesicle. The vesicle produces a series of phialides on the upper half.

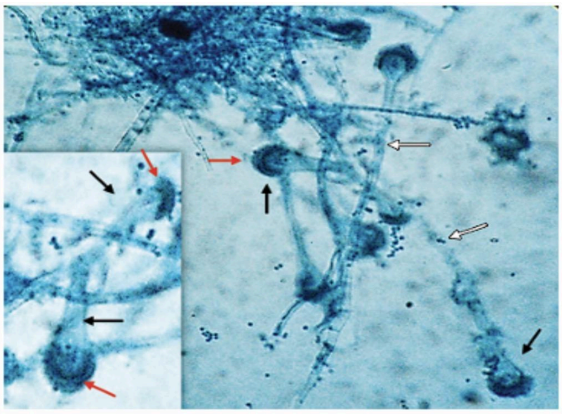


Fig. 4.44: Microscopic morphology of *Aspergillus fumigatus*. Conidiophores are smooth walled, colourless (white arrow), vesicles are flask-shaped (black arrow), phialides uniseriate and are arranged parallel to the conidiophore (inset: Red arrow) [LPCB mount ×200].

Aspergillus flavus

Colony morphology

Colony grows rapidly on Sabouraud's dextrose agar and consists of a close textured basal mycelium which is flat or radially furrowed or wrinkled (Fig. 4.56). Conidial heads are abundant and of intense yellow to yellowish green in colour. Reverse is colourless.

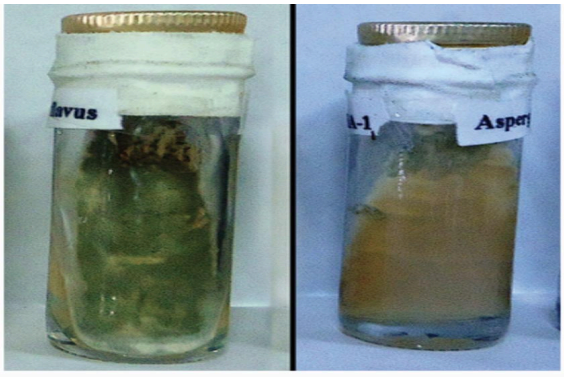


Fig. 4.45: Colony morphology of *Aspergillus flavus* at 28°C for 5–6 days. Colonies grow rapidly as white flocculent at first and gradually become velvety, yellowish green when conidial heads mature and produce conidia, reverse is colourless; (a) obverse, (b) reverse.

Microscopic morphology

Conidiophores

are thick-walled, unpigmented, coarsely roughened, long and ends in a vesicle which is globose to subglobose and produce phialides covering the entire surface. Phialides are biseriate or sometimes uniseriate. Primary phialides are up to 10 μ in length and the secondary phialides are about 5 μ in length.

The conidia are elliptical at first, but later they are mostly globose and echinulate (Fig. 4.46).

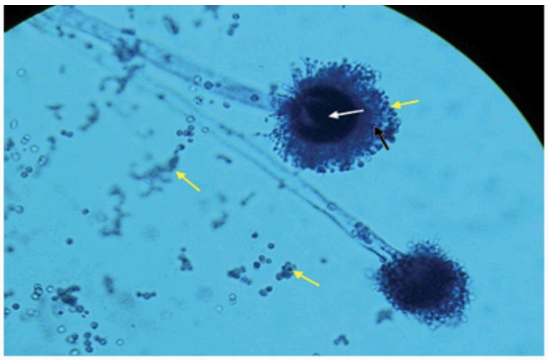


Fig. 4.46: Microscopic morphology of *Aspergillus flavus*. Vesicles (white arrow) are globose, sterigmata biseriate and radiating (black arrow), conidia are round to oval smooth greenish yellow (yellow arrow) [LPCB mount ×400].

Aspergillus terreus

Colony morphology

On Sabouraud's dextrose agar at 25°C to 30°C the colony grows rapidly. The consistency of the colony is floccose to velvety, sometimes furrowed or tufted and the colony conidiates profusely. The massed conidial heads are columnar and the colour is cinnamon-buff to wood brown which is revealed in colony colour. Reverse is usually colourless(Fig. 4.47).



Fig. 4.47: Colony morphology of *Aspergillus terreus* grown on Sabouraud's dextrose agar at 25°C for 2 weeks; (a) obverse is velvety, furrowed, cinamon-buff to wood brown in colour, (b) reverse is dark brown.

Microscopic morphology

The conidiophores are long and slender, smooth and uniform in diameter throughout. The vesicles are hemispherical or dome-like and merge in perceptively with the conidiophore. The phialides are in two series. The primaries are longer and wider than secondaries.

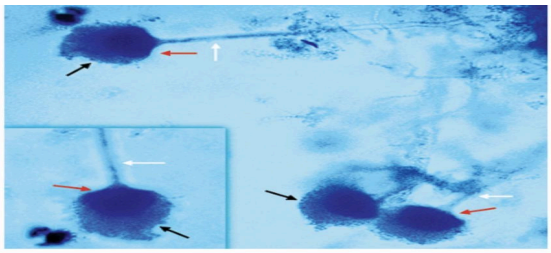


Fig. 4.48: *Aspergillus terreus*. Microscopic morphology showing conidiophores long, smooth and colourless (white arrow), vesicles are hemispherical to subglobose (red arrow), phialides are radiating, arranged in two rows (biseriate, black arrow) [LPCB mount ×400].

HISTOPLASMOSI

Histoplasmosis is a granulomatous disease of worldwide distribution caused by dimorphic fungus Histoplasma capsulatum



Fig. 4.49: Secondary cutaneous lesions in disseminated histoplasmosis in HIV reactive patient. Papular skin lesions (white arrow) are suggestive of histoplasmosis while the papulopustular lesions (black arrow) are very much suggestive of cutaneous manifestation of disseminated blastomycosis.

Fig. 4.50: Cutaneous lesions in blastomycosis. These are papulopustular lesions which gradually become necrotic (black arrow) with some scarring in the center (red arrow).

Clinical forms of histoplasmosis

1. Benign infection

- Endemic histoplasmosis (primary cutaneous disease; 'fungus flu' in adults; summer sickness in children; subclinical disease
- Epidemic histoplasmosis (acute pulmonary histoplasmosis and acute disseminated histoplasmosis)

2. Opportunistic infection

- Chronic pulmonary histoplasmosis (cavitary histoplasmosis; smokers emphysema; colonization of preexisting structural anomalies)
- Disseminating histoplasmosis (fulminant disease in children; fulminant disease of adults associated with immunosuppression)

3. Aberrant fibrosis and hypersensitivity disease

- Histoplasmoma
- Mediastinal fibrosis.

Laboratory Diagnosis

Direct examination

- KOH > negative
- Yeast cells are predominantly within macrophages or monocytes but may appear extracellular because of destruction of phagocytic cells. Yeast cells are ovoid, 2 to 4um in diameter with the bud at the smaller end and budding is characteristically with a ‘narrow base’ (Fig. 4.51).

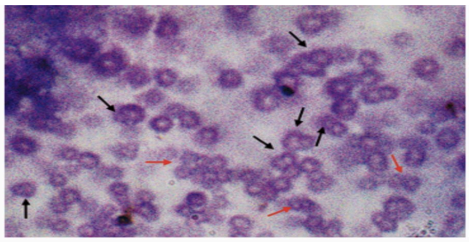


Fig. 4.51: Giemsa stained smear (superimposed) prepared from punch biopsy (skin) material dipped in KOH solution. Broad based budding yeast cells (black arrow) are suggestive of yeast cells of *Blastomyces dermatitidis*. A few yeast cells (red arrow) with narrow based budding are suggestive of yeasts of *Histoplasma capsulatum* [Giemsa stain ×1000].



Fig. 4.52: Colony morphology of *Histoplasma capsulatum* grown on Sabouraud's dextrose agar at 25°C for two weeks. White to buff colonies appear which on repeated transfer produce white dense aerial hyphae.

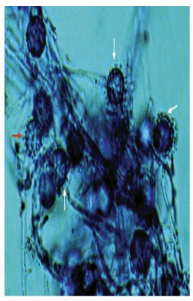


Fig. 4.53: Characteristic macroconidia of *Histoplasma capsulatum*. Large, thick walled, spherical (oc oblong or pear shaped) macroconidia (white arrow) with finger-like projections (red arrow); al 'tuberculate' conidia [LPCB mount ×600].

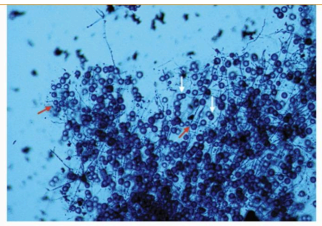


Fig. 4.55: Yeast conversion of *Histoplasma capsulatum* (M→Y). Microscopic morphology of yeast form from culture. Mixture of hyphae in the process of conversion and freely budding yeast cells are seen. cells are small (2–3 μ × 3–4 μ), oval to round and have a narrow neck of budding. The attachment is drawn out as a narrow neck (red arrow). Some broad based budding yeast cells (white arrow) indicate conversion of *Blastomyces dermatitidis*; might be the lesion is caused by dual pathogenic fungi [LPCB ×400].

BLASTOMYCOSIS

- **Blastomycosis** is a chronic granulomatous and suppurative disease having a primary pulmonary stage that is frequently followed by dissemination to the other body site, chiefly the skin and bone.

CA: Blastomyces dermatitidis (a soil saprophyte in nature.)

Laboratory Identification

Direct examination

Examination in a KOH preparation is the easiest and most rapid procedure for diagnosis.

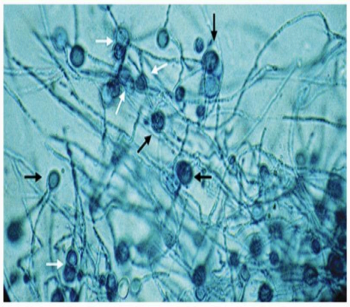


Fig. 4.57: *Blastomyces dermatitidis*. Mycelial form at 25°C. The conidia are of *Chrysosporium* type, ovoid (black arrow) to dumbbell shaped (white arrow) and may vary from 2 to 10 μ in diameter. Conidia are borne on short lateral (white arrow) or terminal branches of the mycelium (black arrow) [LPCB mount ×400].



Fig. 4.58: *Blastomyces dermatitidis*. Typically smooth, round conidia (white arrow) produced on long branched (black arrow) and short lateral unbranched (red arrow) conidiophores [LPCB mount ×200].

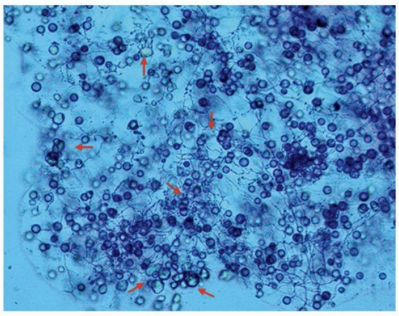


Fig. 4.59: Yeast conversion (M→Y) in *Blastomyces dermatitidis*. Culture mount showing myceli to yeast cells and freely budding yeast cells characteristically having a broad base (red arrow) ×200].

Colonial Morphology

Fluffy white mycelium; others grow slowly as glabrous, tan non-conidiating colonies. Some strains have a dark brown colony consisting of concentric rings that conidiate heavily



Fig. 4.56: Colony morphology of *B. dermatitidis* grown on SDCA (+ 10% glucose) at 37°C for two weeks (M→Y conversion is usually done on blood agar with 10% glucose supplement).

MUCORMYCOSIS

- is a serious fungal infection caused by the agents belonging to the order Mucorales.

The CAUSATIVE AGENTS of mucormycosis in human are:

- Mucor species
- Rhizopus oryzae (arrhizus)
- Rhizopus microspores var. rhizopodiformis
- Cunninghamella bertholletiae
- Lichtheimia corymbifera
- Rhizomucor pusilus
- Apophysomyces elegans
- Cokeromyces recurvatus
- Syncephalastrum racemosum
- Saksenaea vasiformi



Fig. 4.60: Rhinocerebral mucormycosis in a patient with uncontrolled diabetic mellitus of many years duration.



Fig. 4.61: Primary cutaneous mucormycosis over the left gluteal region in a diabetic patient with renal compromization. Necrosis, eschar formation, central ulceration and sloughing are seen. *Rhizopus oryzae* was cultured from the lesion.



Fig. 4.62: Direct mount of punch biopsy from the lesion in the nasal area in a case of rhinocerebral mucormycosis. Sparsely septate broad ribbon-like hyphae with wide angle branching (red arrow) are noticed in this KOH preparation. Black arrow shows columella of the ruptured sporangium [×400].



Fig. 4.63: Direct microscopy of a nasal scrapings after potassium hydroxide digestion in a case of rhino-cerebral mucormycosis. Broad, sparsely septate, thick walled, refractile hyphae (black arrow) are noticed. Some swollen cells and distorted hyphae (red arrow) are also seen [KOH preparation ×400].

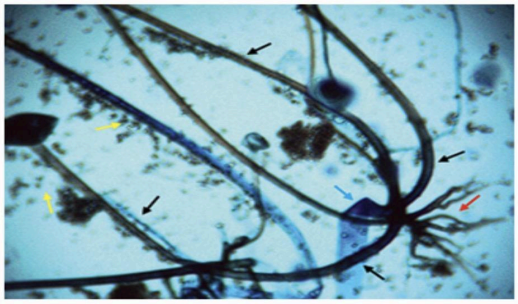


Fig. 4.66: *Rhizopus oryzae*. Sporangiophores are simple or branched (black arrow), rhizoids are yellow-brown (red arrow) and sporangiospores (yellow arrow) are light brown, striated, round to oval sometimes irregular in shape [LPCB mount ×600].



Fig. 4.67: Colony morphology of *Mucor* spec Sabouraud's dextrose agar at 37°C for 48 hours growth is achieved at this temperature. Colon fast growing, white at first and become smok with age. The reverse is cream to yellow; (a) ob (b) reverse.

Mucor Species

Two members of this genus that cause human disease are **Mucor circinelloides** and **Mucor ramosissimus.**

Colony morphology

Colonies are fast growing, smoky gray, up to 2 cm in height; the reverse is yellow to cream coloured and odoriferous

Microscopic morphology

Sporangiophores are of two types—elongate sporangiophores bear large sporangia (40 to 80 µ) and are brown (Figs 4.69 and 4.70), filled with granules, sympodially branched, with tapering branches

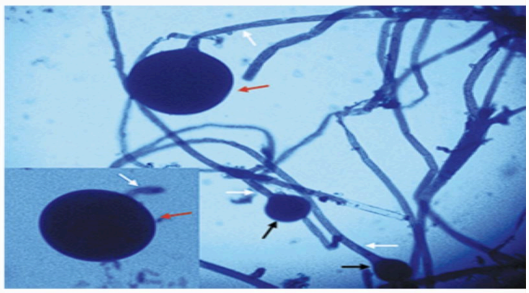
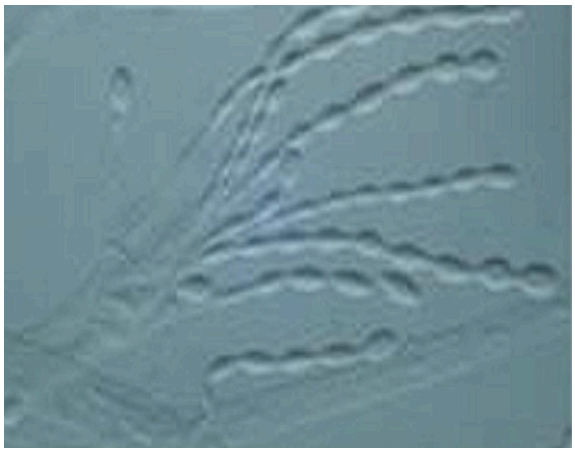


Fig. 4.70: *Mucor* species. Sporangia (black arrov are in the process of maturation. Mature sporangiu (red arrow) are dark brown, spherical and without ar apophysis. Sporangiophores are indicated by whit arrow [LPCB mount ×400].

Paecilomyces lilacinus

Characteristics:

- **Macroscopic examination:**
- Tan, brownish gold, or mauve flat, granular to velvety colonies
- Microscopic examination:
- Spindle shaped conidia
- Spindle: both ends are pointed



Cunninghamella spp.

Characteristics:

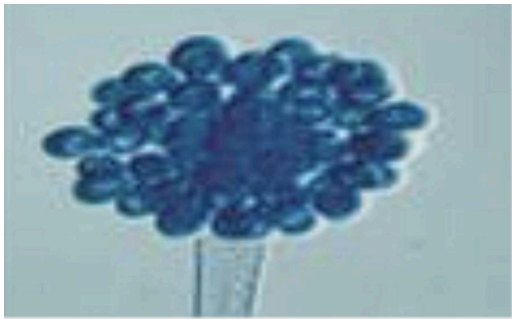
- Recovered from sinuses or other organs during disseminated disease

Macroscopic examination:

o White to gray cottony colonies

Microscopic examination:

o Branching sporangiophores with sporangioles



Fusarium spp.

Characteristics:

- Seen in mycotic keratitis
- Associated with soft contactlenses.

Macroscopic examination:

o rose to mauve to purple to yellow colonies

Microscopic examination:

o **Banana**- or canoe-shaped macroconidia in clusters (sporodoch)



Rhizomucor pusillus

The genus is similar to Mucor but has some poorly developed rhizoids.

Colony morphology

Similar to **Mucor** species. The colony is rapidly growing, smoky gray at first and becomes grayish brown to dark brown with age (Fig. 4.72)

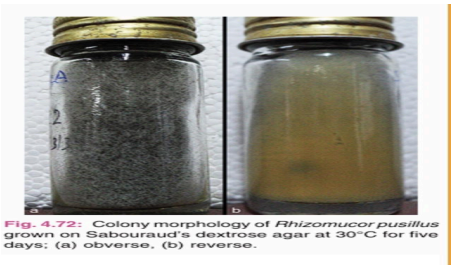


Fig. 4.72: Colony morphology of *Rhizomucor pusillus* grown on Sabouraud's dextrose agar at 30°C for five days; (a) obverse, (b) reverse.

Lichtheimia corymbifera

Colony morphology

The colonies are rapidly growing, floccose, light olive gray coloured. The colour fades in time (Fig. 4.73)



Fig. 4.73: *Lichtheimia corymbifera*. On SDCA a rapidly growing floccose light gray colony appears. Reverse is often light brownish; (a) obverse, (b) reverse.



Fig. 4.76: *Lichtheimia corynbifera*. The columella is exposed after the rupture of sporangium. The columella (white arrow) is 16 to 27 µ in diameter, ovoid to spatulate and have a few spines (red arrow). The spores (yellow arrow) are lying dispersed in the surrounding area. Black arrow shows sporangiophore. [LPCB ×600]

Cryptococcus

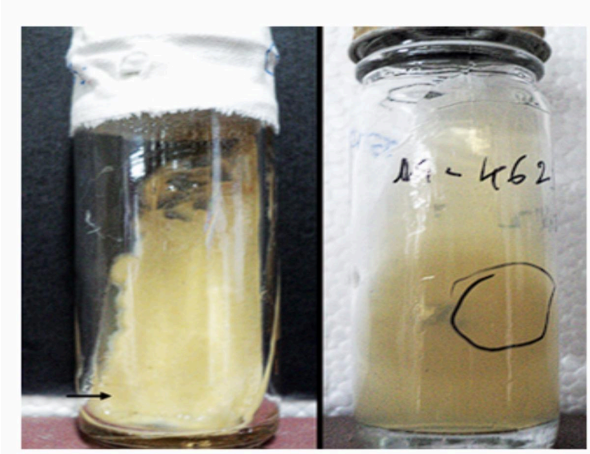


Fig. 4.88: Colony morphology of *Cryptococcus* species on SDCA at 37°C after 3 weeks. Colonies are cream coloured to yellowish, mucoid and may flow to the bottom of the slant (black arrow). The edges are entire and without pseudomycelium; (a) obverse, (b) reverse.

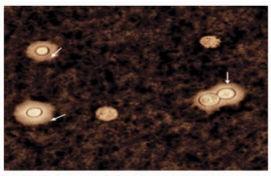


Fig. 4.90: Yeasts of *Cryptococcus neoformans* are seen in wet mount with a drop of India ink. The capsule is so distinctive that it can be easily outlined by negative contrast. The thick polysaccharide capsule appears as a 'halo' surrounding the cell (white arrow). This clinical strain was isolated from a HIV reactive 14 years old boy and was clinically diagnosed as a case of 'meningitis'.

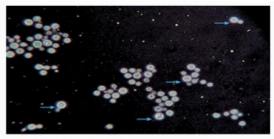


Fig. 4.91: Yeasts of *Cryptococcus neoformans* are seen in wet mount with a drop of India ink. The capsule is not so much distinctive here as it is prepared from a laboratory maintained strain where the capsule has become thinned out due to repeated subculture [×400].

Characteristics:

- Important causes of meningitis, pulmonary disease, and septicemia
- C. neoformans
  - o major cause of opportunistic infection in patients with AIDS

INTRODUCTION TO VIROLOGY

DIFFERENCES BETWEEN VIRUS & CELLS

Table 1. Differences between viruses and cells

Property	Viruses	Cells
Type of nucleic acid	DNA or RNA but not both	DNA and RNA
Proteins	Few	Many
Lipoprotein membrane	Envelope present in some viruses	Cell membrane present in all cells
Ribosomes	Absent	Present
Mitochondria	Absent	Present in eukaryotic cells
Enzymes	None or few	Many
Multiplication by binary fission or mitosis	No	Yes

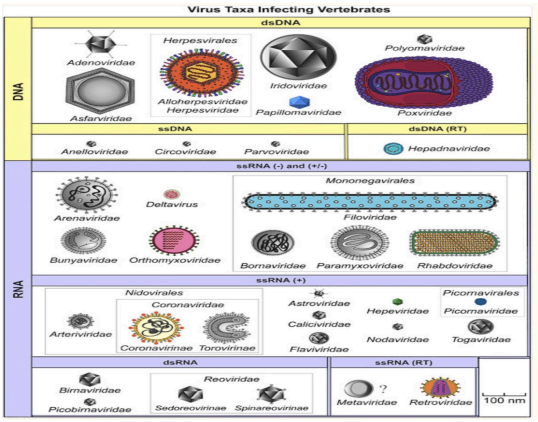
VIRUSES ARE OBLIGATE INTRACELLULAR PARASITES

- They have to enter into a host cell to survive. They cannot survive **outside human cell** in general.
- They must replicate within the host cell.
- Viruses **cannot generate energy or synthesize proteins on their own.** They have to make use of the mechanism of the host cel

STRUCTURE

SIZE AND SHAPE

- Viruses vary in size and shape
- Range from largest protein to smallest cell
- Shape determined by subunits that form protein coat.



VIRAL NUCLEIC ACID

- Viruses contain only DNA or RNA, but not both
- Single or double stranded DNA
- Single or double stranded RNA
- In human cells, RNA is single stranded, but in viruses it can be either single or double stranded

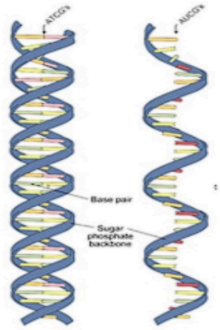


Figure 3. *Double stranded (left) and single stranded (right).*

- **Configuration**: linear or circular similar to bacteria
- **DNA** is always a single molecule
- **RNA** could exist as a single molecule or in several pieces or fragments
- Almost all viruses are haploid (only a single copy of genome

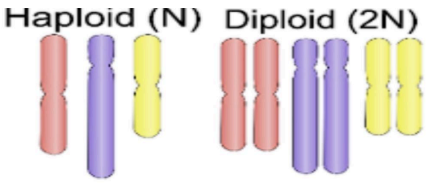


Figure 4. *Haploid vs. Diploid*

➢ **Exception:** Retrovirus (diploid: 2 copies of genome)
- Example: HIV AIDS

VIRAL CAPSID SYMMETRY

- The **nucleic acid** is surrounded by a protein coat called a capsid
- **Capsomeres**: subunits of the capsid
- **Nucleocapsid:** nucleic acid genome and capsid protein
- The arrangement of the capsomeres gives the virus its geometric symmetry

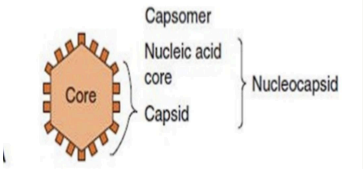
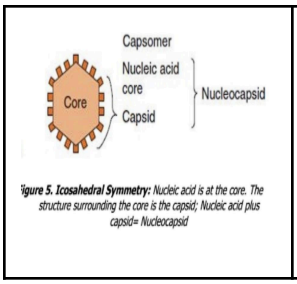


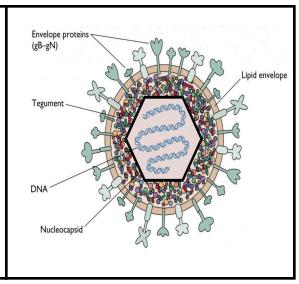
Figure 5. Icosahedral Symmetry: *Nucleic acid is at the core. The structure surrounding the core is the capsid; Nucleic acid plus capsid= Nucleocapsid*

TWO FORMS OF SYMMETRY

1. Icosahedral symmetry

- The capsomeres form a symmetric figure
- Capsomere approximates the outline of a sphere. So it is not exactly round, but its very similar to the outline of the sphere
- Enveloped or naked





2. Helical symmetry (comes from the word helix which implies a coil)

- The capsomeres are arranged in a hollow coil
- Helix or coil: rigid or flexible
- All viruses with helical are enclosed by an envelope
- No naked helical virus

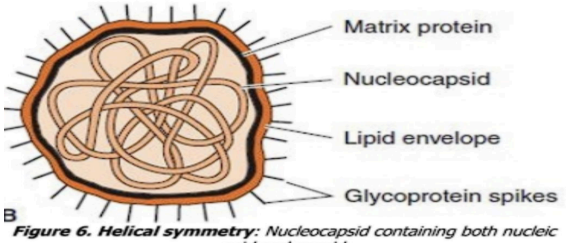
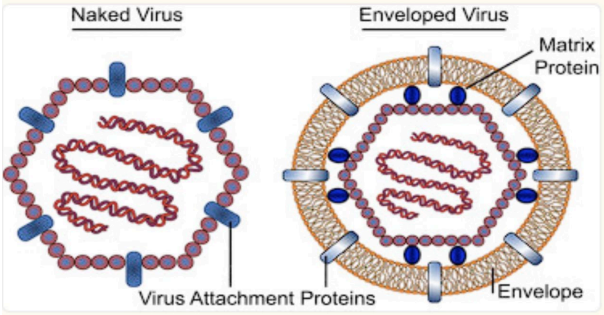


Figure 6. Helical symmetry*: Nucleocapsid containing both nucleic acid and capsid*

VIRAL ENVELOPE

- is a lipoprotein membrane

- **LIPOPROTEIN**- protein part – virus specific
- **GLYCOPROTEIN-** spike like, its function is to attach to the host cell receptor
- **SURFACE PROTEIN –** act as principal antigen that serve as determinants of specificity (serotypes)



CLASSIFICATION OF MEDICALLY IMPORTANT VIRUSES

- Based on chemical and morphologic criteria.

a.Chemical

- Based on the nucleic acid (structure)
- DNA or RNA
- Single-stranded or double stranded

b. Morphologic

- Capsid
- Symmetry (Icosahedral or Helical)
- Envelope (Enveloped vs Naked)

DNA VIRUSES

FAMILY	VIRUS	DISEASE
Papillomavirus	Human Papilloma virus (HPV)	Genital warts Cervical Cancer
Adenovirus	Adenovirus	Respiratory tract infections
Hepadnavirus	Hepatitis B virus	Hepatitis B
Herpesvirus	Herpes simplex	Genital Herpes
	Varicella-zoster virus	Chicken pox Herpes Zoster
	Cytomegalovirus	Congenital abnormalities
	Ebstein-barr virus	Infectious mononucleosis Nasopharyngeal carcinoma
Poxvirus	Smallpox virus	Smallpox
	Molluscum contagiosum virus	Lesion on the skin

RNA VIRUSES

FAMILY	VIRUS	DISEASE
Picorna	Poliovirus	Poliomyelitis
	Rhinovirus	Common cold
	Hepatitis A virus	Hepatitis A
Reovirus	Rotavirus	Acute gastroenteritis (Children)
Flavivirus	Yellow fever virus	Yellow Fever (Jaundice)
	Dengue virus	Dengue Fever
	West Nile Virus	West Nile Fever
	Hepatitis C Virus	Hepatitis C
Togavirus	Rubella virus	German measles
Retrovirus	HIV	HIV/AIDS
	Human T-cell leukemia Virus	Leukemia
Orthomyxovirus	Influenza virus	Influenza
Paramyxovirus	Measles virus	Measles/Rubeola
	Mumps virus	Orchitis
	Respiratory syncytial virus	Respiratory tract infections
Rhabdovirus	Rabies virus	Rabies
Filovirus	Ebola virus	Hemorrhagic fever
	Marburg	
Coronavirus	Coronavirus	SARS SARS-COV 2

VIRAL REPLICATION

Viral replication cycle can be described in 2 ways:

A. Growth Curve

B. Specific events within the cell

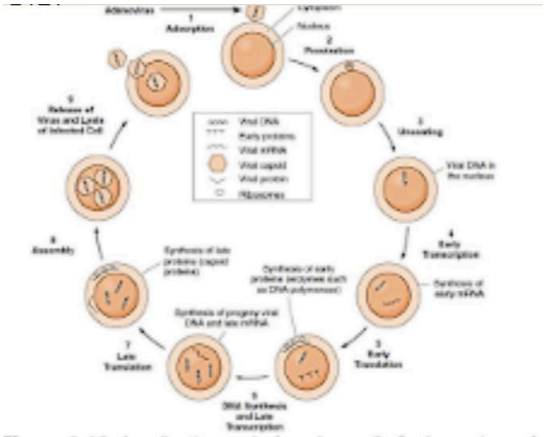


Figure 1. Viral replication cycle (see Appendix for larger image)

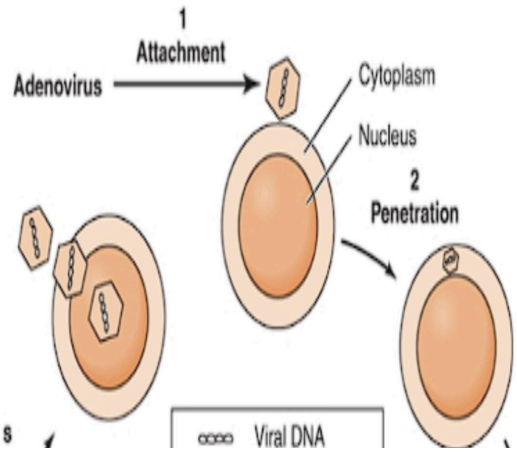
STAGES OF VIRAL REPLICATION

- ATTACHMENT AND PENETRATION

➢ Virus attaches to the cell membrane and penetrates the host cell.mBefore entry, it has to attach aka Adsorption/attachment (**1) first, penetrates the cell membrane** and

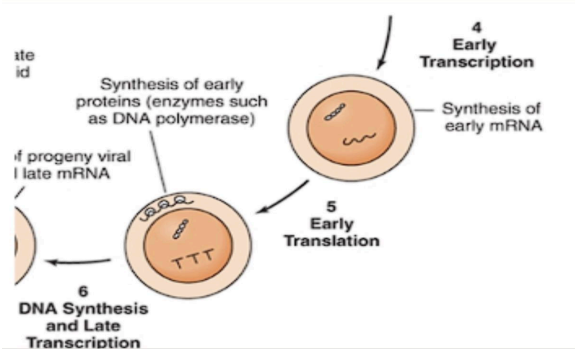
gains entry into the **cell-Penetration (2)**

- ➢ Viral genome is uncoated by removal of the capsid proteins.
- ➢ After uncoating, it will now expose the nucleic acid which finds its way to the

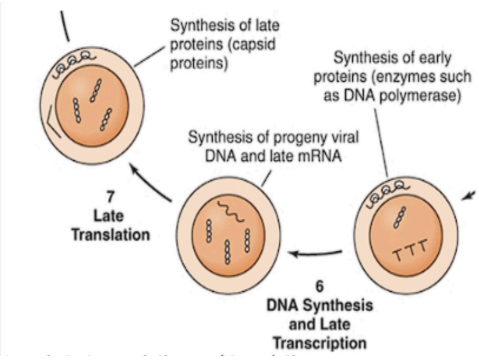


3. Early Viral mRNA Synthesis

- Early mRNA and proteins are synthesized.
- Early proteins are enzymes used to replicate the viral genome.
- In DNA replication, enzymes are required (DNA polymeras)

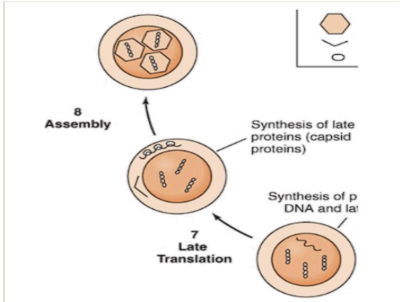


4. Early Viral Protein Synthesis
5. Viral Genome Replication
6. Late Viral mRNA Synthesis
7. Late viral protein synthesis



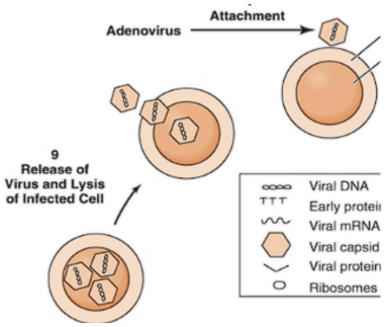
8. Progeny virion assembly

- After all the components are synthesized, assembly occurs.
- The progeny virions are assembled from the replicated genetic materials and newly made capsid proteins
- Synthesis of numerous virus



9. Virion release from the cell

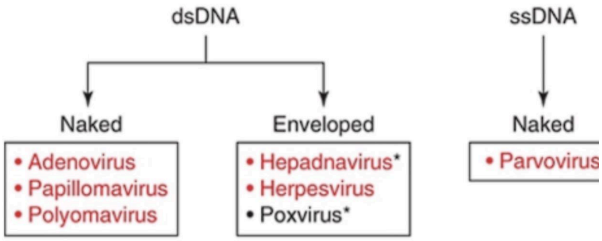
- Progeny virions are then released from the cell.
- Host cell will lyse and death occurs.
- New viruses will again attach and penetrate other cells and the cycle is repeated



REPLICATION -SUMMARY

EARLY EVENTS	Attachment Penetration Uncoating
MIDDLE EVENTS	Gene expression Genome replication
LAST EVENTS	Assembly Release

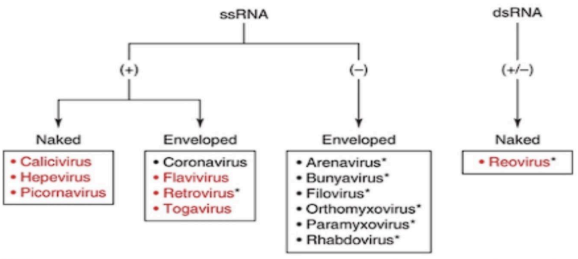
DNA VIRUSES



Red = icosahedral symmetry
(Poxvirus has complex symmetry)

*Viruses labeled with asterisk have a polymerase in the viri

RNA VIRUSES



Red = icosahedral symmetry
(All others have helical symmetry)

* Viruses labeled with asterisk have a polymerase in the virion

CLASSIFICATION OF DNA VIRUSES

Virus Family	Envelope Present	Capsid Symmetry	Virion Size (nm)	DNA MW (×10⁶)	DNA Structure[1]	Medically Important Vir
Parvovirus	No	Icosahedral	22	2	ss linear	B19 virus
Polyomavirus	No	Icosahedral	45	3	ds circular, supercoiled	JC virus, BK virus
Papillomavirus	No	Icosahedral	55	5	ds circular, supercoiled	Human papillomavirus
Adenovirus	No	Icosahedral	75	23	ds linear	Adenovirus
Hepadnavirus	Yes	Icosahedral	42	1.5	ds incomplete circular	Hepatitis B virus
Herpesvirus	Yes	Icosahedral	100[2]	100–150	ds linear	Herpes simplex virus, varicella-z cytomegalovirus, Epstein–Ba
Poxvirus	Yes	Complex	250 × 400	125–185	ds linear	Smallpox virus, molluscum contag

CLASSIFICATION OF RNA VIRUSES

Virus Family	Envelope Present	Capsid Symmetry	Particle Size (nm)	RNA MW (×10⁶)	RNA Structure[1]	Medically Important Viruses
Picornavirus	No	Icosahedral	28	2.5	ss linear, nonsegmented, positive polarity	Poliovirus, rhinovirus, hepatitis A virus
Hepevirus	No	Icosahedral	30	2.5	ss linear, nonsegmented, positive polarity	Hepatitis E virus
Calicivirus	No	Icosahedral	38	2.7	ss linear, nonsegmented, positive polarity	Norovirus
Reovirus	No	Icosahedral	75	15	ds linear, 10 or 11 segments	Rotavirus
Flavivirus	Yes	Icosahedral	45	4	ss linear, nonsegmented, positive polarity	Yellow fever virus, dengue virus, West Nile virus, hepatitis C virus, Zika virus
Togavirus	Yes	Icosahedral	60	4	ss linear, nonsegmented, positive polarity	Rubella virus
Retrovirus	Yes	Icosahedral	100	7[2]	ss linear, 2 identical strands (diploid), positive polarity	HIV, human T-cell leukemia virus
Orthomyxovirus	Yes	Helical	80–120	4	ss linear, 8 segments, negative polarity	Influenza virus
Paramyxovirus	Yes	Helical	150	6	ss linear, nonsegmented, negative polarity	Measles virus, mumps virus, respiratory syncytial virus
Rhabdovirus	Yes	Helical	75 × 180	4	ss linear, nonsegmented, negative polarity	Rabies virus
Filovirus	Yes	Helical	80[3]	4	ss linear, nonsegmented, negative polarity	Ebola virus, Marburg virus
Coronavirus	Yes	Helical	100	10	ss linear, nonsegmented, positive polarity	Coronavirus
Arenavirus	Yes	Helical	80–130	5	ss circular, 2 segments with cohesive ends, negative polarity	Lassa fever virus, lymphocytic choriomeningitis virus
Bunyavirus	Yes	Helical	100	5	ss circular, 3 segments with cohesive ends, negative polarity	California encephalitis virus, hantavirus
Deltavirus	Yes	Uncertain[4]	37	0.5	ss circular, closed circle, negative polarity	Hepatitis delta virus

PATHOGENESIS OF VIRAL INFECTIONS

The ability of viruses to cause disease can be viewed on two levels

1. The changes that occur within individual cell
   - Viruses are intracellular pathogen

2. The process that takes place in the infected patient

THE INFECTED CELL

– there are four main effects of virus infection on the cell

1. CELL DEATH

- Probably due to inhibition of macromolecular synthesis
- Inhibition of host cell protein synthesis

o Primary mechanism

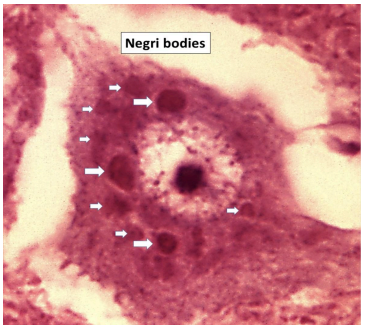
- Inhibition of DNA and RNA synthesis

o Secondary mechanism

- Synthesis of cellular proteins of the host is inhibited
- Viral protein synthesis still occurs
- Infected cells frequently contain inclusion bodies
- Contain viral proteins or viral particles
- Characteristic intranuclear or intracytoplasmic location and appearance
- Exclusion of inclusion bodies

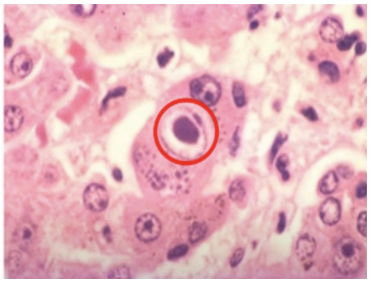
➢ **Negri bodies**

- Eosinophilic cytoplasmic inclusions
- Seen in rabies virus infected neurons
- When a dog dies, the brain of the dog is examined by public health authorities to determine if the dog has rabies



- **CMV infected cells**

o Owl's eye inclusion
o Seen in the nucleus



2. FUSION OF CELLS TO FORM MULTI-NUCLEATED CELLS

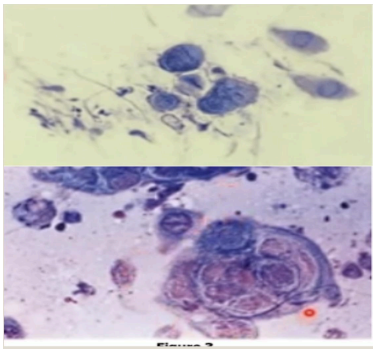
- Fusion of virus-infected cells produces multinucleated giant cells
- Multiple nuclei within a giant cells

➢ **Typical in some family of viruses**

- Herpesviruses
- Paramyxoviruses

Mechanism:

- Insertion of viral protein into cell membrane
- Leads to cell membrane changes
- Eventually fusion of adjacent cell occur



CYTOPATHIC EFFECT

- A hallmark of viral infection of the cell is the cytopathic effect (CPE)
- Change in the appearance of the infected cell
- Rounding and darkening of the cell
- Leads to lysis (disintegration) or giant cell formation
- Detection of virus in a clinical specimen is based on the appearance of CPE in cell culture

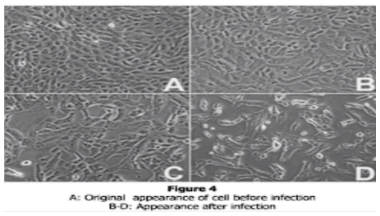


Figure 4
A: Original appearance of cell before infection
B-D: Appearance after infection

3. MALIGNANT TRANSFORMATION

➢Infection with certain viruses causes malignant transformation

- Unrestrained growth
- Prolonged survival

Morphologic changes (polymorphism, irregular nuclei)

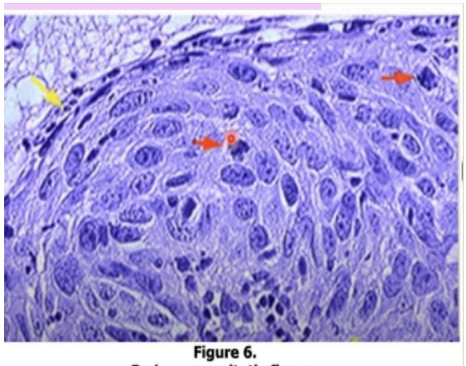


Figure 6.
Red arrow: mitotic figures

4. NO APPARENT CHANGE

- Least common effect
- Infection can occur without morphologic or functional changes
- Symbiotic relationship of host and viruses
- Cell survives and multiplies despite viral replication

THE INFECTED PATIENT

A. TRANSMISSION AND PORTAL OF ENTRY

- **Pathogenesis** in the infected patient involves the following
- Transmission of the virus and entry into the host
- Replication of the virus and damage to cells
- Spread of the virus to other cells and organs
- **The immune response:** host defense
- **Can cause:** Persistence of the virus in cells or organs
- The stages of a viral infection are the same as those for a bacterial infection
- Incubation period (asymptomatic)
- Time of infection to development of s/s
- Prodromal period (nonspecific symptoms)
- Fever, body malaise, anorexia, etc
- Specific-illness period
- Recovery period (illness wanes)
- Some are self limiting
-

> Portals of entry are varied: depends on the specific virus

- Respiratory tract
- GIT
- Skin
- Genital tract
- Blood
- Transplacental

➢**Person to person spread occurs by transfer to:** (HORIZONTAL TRANSMISSION)

- Respiratory secretions
- Saliva
- Blood
- Semen
- Fecal contamination of food and water
- The transfer of blood can transmit various viruses
- Tranfusion
- Sharing of needles during IV drug use

- Screening of donated blood has reduced the risk of infection

VERTICAL TRANSMISSION

- Transmission can also occur between mother and offspring
- In utero across the placenta
- At the time of delivery (passage thru infected birth canal)
- Breastfeeding
- Animal to human transmission can take place

Directly	Indirectly
> Bite of a reservoir host	> Bite of insect vector

Most viral infections are either

1. Localized or close to the portal of entry

- Example:
- **Common cold: Rhinoviruses** – remain localized to the upper respiratory tract
- **Influenza: Influenza virus** – remains in the upper and lower respiratory tract

2. Spread systemically throughout the body

- Example
- **Paralytic poliomyelitis:** caused by **polio virus-** One of the best understood systemic infections

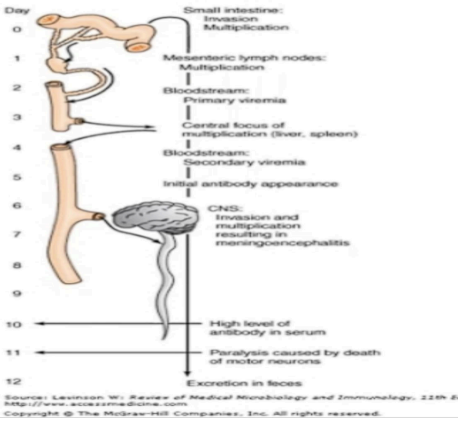


Figure 7. Showing the pathogenesis of poliomyelitis. How is poliomyelitis acquired or what is the portal of entry or transmission? It's not respiratory, it's in fact, **fecal-oral.** The virus is ingested, that is why the vaccine, OPV (Oral Polio Vaccine), is also oral. The virus will find its way into the small intestines where it invades and multiplies. And then from there, it will spread to the mesenteric lymph nodes and to the blood stream. When it reached the bloodstream for the first time, then this is called **primary viremia**. From there, it will reach the central focus of multiplication which is the **liver** and the **spleen**. From there it goes again to the blood stream leading to a **secondary viremia**. And then it is only then when the virus will reach the **CNS**. There will be, again, invasion and multiplication, resulting in a **meningoencephalitis** and then **affecting the spinal cord, leading to paralysis caused by the death of motor neurons.** So you see we have different organ systems involved and the spread through the blood stream

Pathogenesis and Immunopathogenesis of certain viral infections

- Death of virus infected cells results in loss of function and leading symptoms of diseases.

For example:

Poliovirus kills the motor neurons Results in paralysis of muscles which is manifestation of poliomyelitis

Envelope glycoproteins of Ebola virus

- Cause damage to vascular endothelial cells
- Leads to Hemorrhage
- Ebola virus causes hemorrhagic fever
- Some diseases are not caused by the virus damaging or killing infected cells but they are caused by other mechanisms. Like
- **Rotavirus-infected** enterocytes produce cytokines
- Causes acute gastroenteritis specially in children
- Cytokines will in turn stimulate the enteric neurons

- This will lead to lead to excess fluid and electrolyte secretion into the bowel lumen.

Clinical manifestation: Diarrhea

- It's not the killing of the enterocyte but it is due to the chemical mediators, these are certain cytokines. It's not a direct damaging or killing of the cells.

There are other diseases in which cells are killed by immunologic attack. Exemplified by:

- Hepatitis A, B, and C viruses
- There will be recognition of the viral antigens on the hepatocyte surface by cytotoxic T cells.
- Cause Damage to the hepatocytes. There is an immunologic mechanism involved, we have the cytotoxic T cells and those are the ones that cause damage to the hepatocytes.
- **Measles virus**
- Infect vascular endothelium in the skin. The small capillaries in the skin
- This will trigger an attack by the cytotoxic T cells.
- **Manifestation**: skin rash brought about by damage to the small capillaries/blood vessels in the skin. So you should be able to explain why there is that maculopapular rash seen in patient with measle

Virus-antibody-complement complexes may form and deposit in tissues.

- antigen-antibody complement complexes
- Hepatitis B virus infection
- The immune complexes form (virus-antibody complement involving the complement system/factors). The immune complexes will form and this will deposit in the tissues, in the joints.
- Leading to Arthritis (early stage of the disease). It's a virus that infects hepatocytes but because of the immune complexes, you should be able to explain why there can be arthritis (inflammation of the joints)

VIRULENCE

- Strains of viruses differ greatly in their ability to cause disease.
- Attenuated viruses:
- Mutated sufficiently
- Such that they have lost the ability to cause disease
- We still make use of them because these are used in vaccines
- Very low virulence but are able to trigger the immune system to produce antibodies.

EVASION OF HOST DEFENSES

- There are some viruses that have a particular talent, that is to evade or to escape the host defense.
- Viruses have several ways by which they evade host defenses.
- This phenomenon is known as immune evasion

Mechanisms include:

- Cytokine decoys
- Virokines
- Multiple antigenic types EX. RHINOVIRUS, INFLUENZA, HIV, HCV

THE VIRUSES

VIRUSES TO BE CONSIDERED	
DNA Viruses	
Family	
Adenoviridae	*Bunyaviridae*
Hepadnaviridae	*Caliciviridae*
Herpesviridae	*Coronaviridae*
Papillomaviridae	*Filoviridae*
Parvoviridae	*Flaviviridae*
Polyomaviridae	*Orthomyxoviridae*
Poxviridae	*Paramyxoviridae*
RNA Viruses	*Picornaviridae*
Family	*Reoviridae*
Arenaviridae	*Retroviridae*
Astroviridae	*Rhabdoviridae*
	Togaviridae
	Other Infectious Agents
	Prions

DNA VIRUS

Family	Viral Members
DNA Viruses	
Adenoviridae	Human adenoviruses
Hepadnaviridae	Hepatitis B virus
Herpesviridae	HSV types I and II, VZV, CMV, EBV, human herpes viruses 6, 7, and 8
Papillomaviridae	Human papilloma viruses
Parvoviridae	Parvovirus B-19
Polyomaviridae	BK and JC polyomaviruses
Poxviridae	Variola, vaccinia, orf, molluscum contagiosum, monkeypox viruses

RULES:

1. All are dsDNA except Parvo and Hepadna.
2. All are icosahedral or cubic except Poxviridae
3. All are enveloped except PPAP.

- P (Parvoviridae)
- P (Polyomaviridae)
- A (Adenoviridae)
- P (Papillomaviridae)

4. All multiply in nucleus except Poxviride

ADENOVIRUS

TABLE 65-3 Adenoviruses

Family	*Adenoviridae*
Common name	Adenovirus
Virus	Adenovirus
Characteristics	Double-stranded deoxyribonucleic acid (DNA) genome; icosahedral capsid, no envelope; approximately 50 human serotypes
Transmission	Respiratory, fecal-oral, and direct contact (eye)
Site of latency	Replication in oropharynx
Disease	Pharyngitis, pharyngoconjunctival fever, keratoconjunctivitis, pneumonia, hemorrhagic cystitis, disseminated disease, and gastroenteritis in children
Diagnosis	Cell culture (HEp-2 and other continuous human epithelial lines), enzyme immunoassay (EIA) for gastroenteritis serotypes 40-41
Treatment	Supportive
Prevention	Vaccine (adenovirus serotypes 4 and 7) for military recruits

6 Groups:	
Group	**Serotype**
A	12, 18, 31
B	3, 7, 11, 14, 16, 21, 34, 35, 50
C	1, 2, 5, 6
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51
E	4
F	40, 41

> medium-sized (70-90 nm)

- STABILITY/VIABILITY: for weeks at variable temperatures on surfaces and in liquid solutions
- IP for respiratory disease: 2-14 days
- Vaccine currently available

LABORATORY DIAGNOSIS:

SPECIMEN: RESPIRATORY SECRETIONS, STOOL

EPITHELIAL CELL LINES: A-549, HEp-2, and He-La cells (growth in 2 -5 days

RESULT: Adenovirus produces a characteristic grapelike cluster cytopathic effect (CPE)

CONFIRMATORY TEST: indirect fluorescent antibody (IFA) technique or enzyme immunoassay
(EIA)
NUCLEIC ACID TESTING / PCR

DISEASE

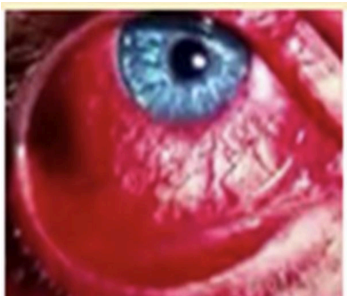
- Causes upper and lower respiratory diseases

a. URTI

- Pharyngitis
- Pharyngoconjunctival fever
- Acute respiratory disease
- Common cold

b. LRTI

- Pneumonia
- Bronchitis
- Enteric strains can cause diarrhea or acute gastroenteritis
- Keratoconjunctivitis
- Hemorrhagic cystitis

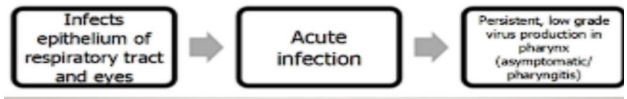


There are Two Genera Under Adenoviridae:

1. **Aviadenovirus-** They are infecting birds
2. **Mastadenovirus**- These are the one infecting mammals including humans - Recently, we have almost 52 serotypes, and human adenovirus are grouped. This 52 serotypes are divided into several groups (A, B, C, D, E, and F)

- The basis of classification is based on their capability to infect a certain location, and similarities in their characteristics.
- Were first isolated from adenoids surgery removed from children in 1953.
- Target cell: Epithelial cells of the respiratory tract, eyes, gi, the urinary bladder & liver

PATHOGENESIS

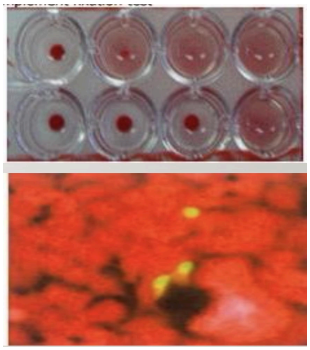


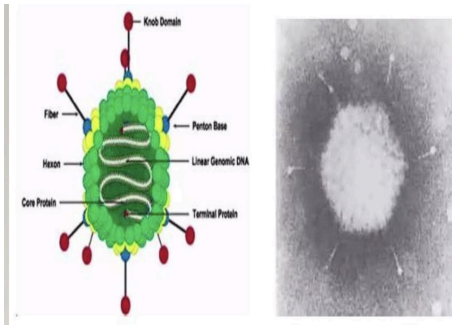
Immunity based on the neutralizing antibody is type specific and lifelong

- Can cause latent infection in the adenoidal and tonsillar tissues of the throat
- Virus in quiescent causes reactivation or replication
- Due to immunosuppression or exposure to inciting agent

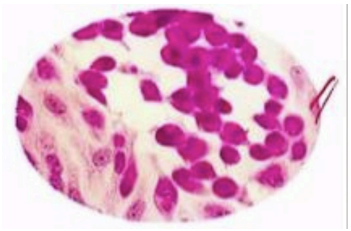
LABORATORY DIAGNOSIS:

1. Cell culture: Gold Standard
- The virus produces cytopathic effect (CPE)
2. Complement Fixation
3. Fluorescent Antibody testing
4. Serology
- Antibody titer rise in convalescent phase serum is diagnostic





These epithelial cells have a specific receptor called Coxsackie adenovirus receptors



CPE - rounding and clustering of cell with refractile intranuclear inclusion bodies

Infectious Period

- While they are symptomatic
- **Latency site**: adenoid, tonsils, lymphoid tissues

PREVENTION

- The live vaccine against types 3, 4, 7 is used in the military to prevent pneumonia
- The vaccine is given orally instead of parenterally

PAVOVIRIDAE

TABLE 65-16 Parvoviruses

Family	*Parvoviridae*
Common name	Parvovirus
Virus	Parvovirus B-19
Characteristics	Single-stranded deoxyribonucleic acid (DNA) virus; icosahedral capsid, no envelope; parvovirus B-19 is the only known human parvovirus.
Transmission	Close contact, probably respiratory
Disease	Erythema infectiosum (fifth disease), aplastic crises in patients with chronic hemolytic anemias, and fetal infection and stillbirth
Detection	Serology, polymerase chain reaction (PCR), histology
Treatment	Supportive
Prevention	Avoid contact.

TABLE 31-1 Important Properties of Parvoviruses

Virion: Icosahedral, 18–26 nm in diameter, 32 capsomeres
Composition: DNA (20%), protein (80%)
Genome: Single-stranded DNA, linear, 5.6 kb, MW 1.5–2.0 million
Proteins: One major (VP2) and one minor (VP1)
Envelope: None
Replication: Nucleus, dependent on functions of dividing host cells
Outstanding characteristics: Very simple viruses Human pathogen, B19, has tropism for red blood cell progenitors One genus contains viruses that are replication-defective and require a helper virus

MW, molecular weight.

Figure 17. Important Properties of Parvoviruses

- Simplest DNA animal viruses (ZOOPATHIC)
- Single stranded
- autonomously replicating
- Parvovirus B19

- One of the few species infecting human
- Parvovirus B19's target are the immature RBCs under the erythroid lineage.
- **Infection**: **Erythema Infectiosum - A.K.A. 5th Disease/Slap Cheek Syndrome**.

- Complications caused by **Parvovirus B19:**

- Polyarthralgia-arthritis syndrome in normal adults
- Aplastic crisis in patients with hemolytic disorders
- Chronic anemia in ICP individuals
- Fetal death



igure 9. **Slapped cheek syndrome** produces red rash on the cheek

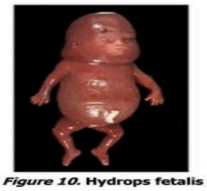


***Figure 10.* Hydrops fetalis**

Proteins

- VP1 – major coating protein
- VP2 – minor coating protein

Envelope

- None, this is a naked virus
- The stability is maintained even at pH 3-9 (wide-range)*
- In terms of heat: Can still survive/be stable at 56C for 30 minutes

Replication

- Nucleus, dependent on functions of dividing host cells

• Inactivated by:

- Formalin
- Beta-propiolactone
- Different oxidizing agents

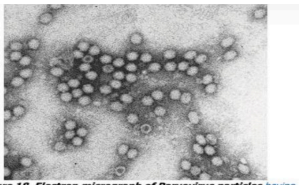


***Figure 18. Electron micrograph of Parvovirus particles** having circular appearance*

1. Parvovirinae

- Usually infecting vertebrates

Three Genera:

1. **Parvovirus**

• Feline Panleukopenia virus & Canine parvovirus

2. **Erythrovirus** • Human parvovirus B19

- 3 strains: Type 1, 2, 3

▪ Type 1: Parvovirus B19
▪ Type 2: strain k71
▪ Type 3: strain V9

3. **Dependovirus**

- **Parvovirus and Erythrovirus:** autonomously replicating viruses
- **Dependovirus:** genera with members that are defective (very dependent on the presence of helper viruses for them to replicate).

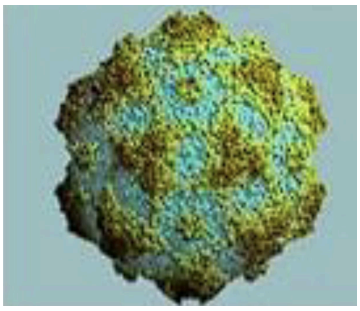
Example of helper virus: Adenovirus and Herpesvirus

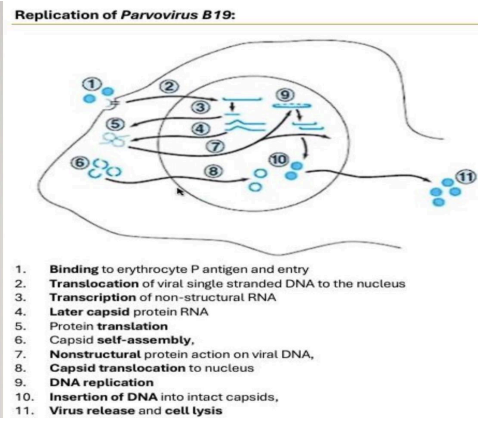
2. Densovirinae

- infects insects

Characteristics of Parvovirus B19:

- 22-26 nm
- Single stranded DNA genome
- Icosahedral & naked
- Capsid is made up of two or three proteins (VP1: major, VP2: minor)
- Infect human as well as rodents, dogs and pigs (zoopathic)
- The B19 name of this originated from a serum sample number. B: panel number, 19: number of the well where the virus was first isolated. This was first isolated and observed by electron microscope.
- Resistant to reagents like acids, bases as its pH ranges from 3-9.
- It can also tolerate less than 56C.
- In terms of the specific antigen in the immature RBCs: P antigen - can be seen in the virus; requires specific receptor from the immature RBCs





Pathogenesis of Parvovirus B19:

- Immature cells in the erythroid lineage
- Major sites of virus replication:

Adult marrow

- Hematopoiesis

Blood cells

- Peripheral circulation: reticulocytes (<1%)

Fetal liver

- Interrupted red cell production
- Transient anemia in patients with parvovirus B19
- Chronic anemia in ICP and not just transient

•Rash associated with erythema infectiosum
• Virus-specific IgM and IgG antibodies

Route of Spread:

Spread

- RT
- Parenteral (the use of needles, may be blood products: whole blood, packed RBC, washed RBCs as long as it has RBCS).

Vertical (mother to baby)

- Short period of viremia before rash appears
- Manifestation: **Erythema Infectiosum**

Clinical manifestation:

- appearance of rashes
- Incubation period: **12-18 days**

Infectious period:

- Prodromal phase

- During this time, there is already viremia Example, donating blood during this time.
- After the appearance of rashes, the route is in the respiratory tract already.
- So, from the end of the incubation period, up to the appearance of rash, the patient is infectious.

Clinical Findings of Parvovirus B19:

Primary infection

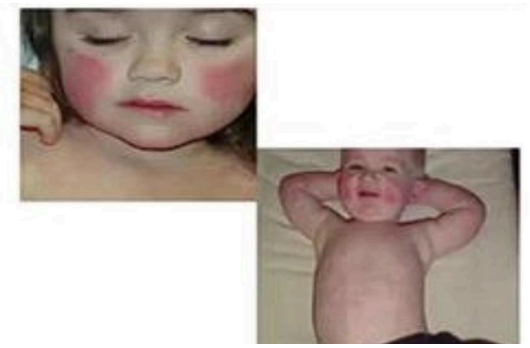
1. Erythema Infectiosum- Most common manifestation in human

Incubation period:

- 1-2 or 3 weeks (12-18 days)
- Viremia occurs 1 weak after infection & persist for about
- 5 days - 50% of infections are **asymptomatic -** Symptomatic infections
- **Biphasic illness -** This has 2 phases:

A. Fever, malaise, URT infection

- The viral shedding (viral exit) is in the URT, thus the route of spread is also through respiratory tract
- IgM increases then IgG 15 days post infection



B. Maculopapular rash

- Occurs when the patient is exposed to sunlight/any form of heat.
- Persist for 17 days after incubation period
- Rash will disappear 2-4 days after exposure

Complications of Parvovirus B19:

- Patient is immunocompetent: transient anemia
- Transient fall in RBC
- Complications occur due to manifestations of severe anemia of an aplastic nature

Infections in Pregnancy:

- Fetal transmission

- Fetal loss (miscarriage due to severe fetal anemia

Hydrops fetalis

- Most severe fetal complication
- Due to fetal edema and ascites
- 2-3% of infected women
- Managed by intra-uterine blood transfusion

Infections in Immunocompromised Patients:

- Chronic parvovirus infection
- Body is not capable of getting rid of the virus immediately
- Chronic intractable aplastic anemia

Infections in Patients with Haemolytic Anemia

• Aplastic crisis

- The bone marrow no longer produces RBCs
- Once the immune response is already mounted, the patient will recover.
- In aplastic crisis, there will be hematologic response. Usually within a week, the reticulocytes increases again

Clinical Indication	Specimen	Test	Significance	Essential Inf for the lab
Diagnosis of acute parvovirus infection	Serum	Parvovirus IgM	Positive result denotes recent infection	Clinical symp date of onset, history of any factor (e.g., p immunocomp or aplastic cri
Diagnosis of acute infection in the immunocompro mised and in patients with aplastic crisis	Serum	Parvovirus DNA PCR	Positive result indicates recent or chronic infection. Specific IgM may be negative in immune-compromised patients due to poor immune response.	Clinical detail symptoms wi factors if ant, onset
Diagnosis of fetal infection	Cord blood by cordocentesis	Parvovirus DNA PCR and IgM if fetus is more than 20 weeks	Positive IgM and/or PCR indicates fetal infection	Gestational a fetus, presen hydrops
Check immune status	Serum	Parvovirus IgG by EIA	A positive IgG in the absence of parvovirus-specific IgM indicates past infection.	History of and recent contac risk factors fo infection.

Treatment of Parvovirus B19:

- No specific treatment, only supportive treatment*

Pregnant women

- Intra-uterine blood transfusion

Immunocompromised patients

- Blood transfusion, intravenous IgG against parvovirus B19

Haemolytic anemia

- Blood transfusion, intravenous IgG against parvovirus B19

Prophylaxis

- No specific prophylaxis or vaccines (for humans wala, but for animals meron na)

Infection control

- Patients should be isolated with respiratory precaution

Human bocavirus (HBoV)

- Described in 2005
- Closely related to bovine parvovirus and canine minute virus of Bocavirus of Parvoviridae
- Co-infection with RSV and human metapneumovirus

Manifestations:

- Upper RT infections
- Cough, rhinorrhea, fever, difficulty in breathing, diarrhea, conjunctivitis, and rash

---

SPECIE: HUMAN PAPILLOMA VIRUS
GENUS: PAPILLOMA VIRUS
FAMILY: PAPILLOMAVIRIDAE
Virion: Icosahedral, 55 nm
Composition: protein (90%), dsDNA (10%)
Genome: dsDNA
Envelope: None
Replication: Nucleus

Outstanding characteristics:

- Stimulate cell DNA synthesis
- Restricted host range and tissue tropism

Restricted: it means that this is localized; there's no systemic manifestations

- This is restricted only to the site of infection or to the tissue that is infected
- This will only be on the skin or mucous membrane; there's no systemic spreading
- Significant cause of human cancer
- Viral oncoproteins interact with cellular tumor suppressor protein

TABLE 65-14 Papillomaviruses

Family	*Papovaviridae*
Common name	Papillomavirus
Characteristics	Double-stranded DNA genome; icosahedral capsid, no envelope; includes papilloma viruses
Virus	Human papilloma virus (HPV)
Characteristics	Contains more than 200 deoxyribonucleic acid (DNA) types
Transmission	Direct contact, sexual contact for genital warts
Site of latency	Epithelial tissue
Disease	Skin and genital warts, benign head and neck tumors, anogenital warts
Diagnosis	Cytology, DNA probes
Oncogenic	Cervical and penile cancer (especially HPV types 16 and 18)
Treatment	Spontaneous disappearance the rule; surgical or chemical removal may be necessary
Prevention	Avoid contact with infected tissue, vaccination.

Route of Spread

- Close contact
- Sexual
- genital warts
- this could be hetero/homosexual
- **Heterosexual**: from penis to vagina
- **MSM**: Men having sex with men; this is anorectal.

Vertical

- laryngeal papilloma & warts
- not transmitted cross placental
- this will be transmitted vertically during the delivery
- the baby should pass through the birth canal; there should be a contact with infected body part this is after birth not like in Parvo, wherein it is during the gestational period

Direct contact with infected material

- common skin warts
- from borrowed items that are infected



Figure 1. **Verruca vulgaris**: warts on the extremities and trunk

Figure 2. **Condyloma acuminata** (genital warts) in women and men

Incubation Period

Warts

- 3 months to an infected partner
- after exposure there's reactivation
- Cancer
- Latency period of several years

Infectious Period

- When lesions are present
- Asymptomatic shedding from genital tract may occur

At Risk Groups

• Both sexes
• All age groups

Pathogenesis

Skin

- Common warts
- Small in size, occur in large numbers anywhere in the body, have roughened surface

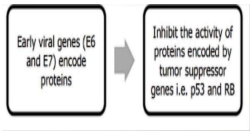
Complaint: cosmetic appearance

- Also known as Verrucae vulgaris
- Genotypes: HPV 1, 2, 4
- Affected areas: hands and feet
- Rough and elevated



Flat warts - Flat and smooth - Generally affect children - Also known as Verrucae planae o Genotypes: HPV 6, 11 - Common in pregnant women or in children* - Flat but it's still a wart





Mucus membrane

- Genital warts: 90% of this is caused by 6 and 11
- Also known as **Condylomata acuminata**
- **Most common sexually transmitted infection (STI) -** Commonly occur in association with other STI
- Papules or papillomatousAffected areas in men:
  - Penis, urethra
  - **Penis:** specifically affects the glans and prepuce

▪ **Anus and rectum:** those who practice MSM or anorectal sex

Affected areas in women:

▪ Vulva, vagina, cervix, anus and perineum

• **Laryngeal warts -** occur in mouth and pharynx

- small children due to vertical transmission



Carcinoma

- **Cervical cancer -** 70% due to genotype 16 and 18 and the rest to other genotypes (31, 33, and 25) - HPV types 16, 18, 31, 33, 3

LABORATORY DIAGNOSIS

Diagnosis is on the basis of

- Clinical history
- Appearance of the lesion on the examination
- The primary consideration is that we're trying to prevent the transmission of the infection
- Other than infecting other people, it can also infect the other areas of the skin

PCR

- Not recommended for routine diagnosis of genital warts
- Most sensitive
- Performed if you need to identify the serological typing or the strain; if not, you can just proceed with the clinical history and appearance of the lesion

You can perform this by getting a sample from the infected areas:

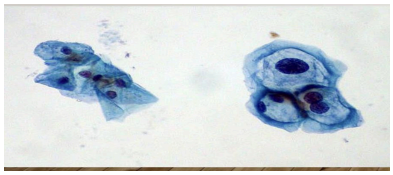
- **Cervix**: cervical swab
- **Lesion**: biopsy of the lesion



Figure 5. **Microscopic appearance of Verruca vulgaris.** Pathologic features: Hyperkeratosis, acanthosis and papillomatosis

Pap Smears

- Koilocytes can be observed by doing Pap smears
- This is called "**pap smear"** since Papanicolaou stain is used
- specimen from the cervix of the females
- If the females are already at the age of 35 years old and above, pap smear is already included in the routine physical examination since this is one of the screenings that we use to identify the presence cervical cancer



Koilocytes: cells with perinuclear clearing accompanied by an increased density of the surrounding rim of cytoplasm

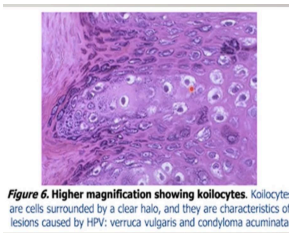
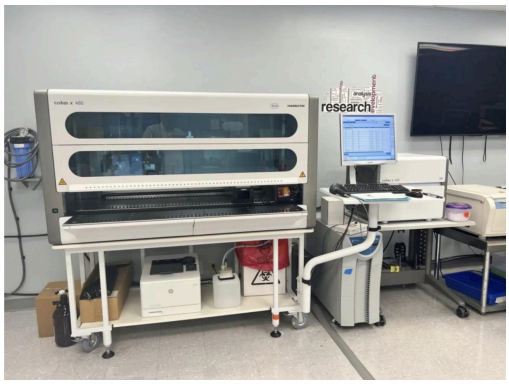


Figure 6. **Higher magnification showing koilocytes.** Koilocyte are cells surrounded by a clear halo, and they are characteristics of lesions caused by HPV: verruca vulgaris and condyloma acuminata

MOLECULAR DIAGNOSIS: DNA HYBRIDIZATION TESTS

- A polymerase chain reaction (PCR)-based test using the Cobas 4800 system is approved to detect the presence of the DNA of 14 high-risk genotypes, including types 16 and 18
- Virus isolation and serologic tests are not done because the diagnosis is often obvious on physical examination, so it is easily recognized



TREATMENT

- Lesions are self-limiting
- Surgical treatment with:

- Podophylin
- Liquid nitrogen
- Salicylic acid

Cervical cancer

- Management by the gynecological oncologist
- All current therapy acts to remove the region of the wart.
- 80% Trichloroacetic acid
- Liquid nitrogen
- S-fluoroacil
- Intralesional interferon
- Laser
- Retinoids

• Cold scalpel excision
• **Podophyllin** - cytotoxic agent
•Electrocautery - commonly available in dermatological clinics together with lassuch as liquid nitrogen

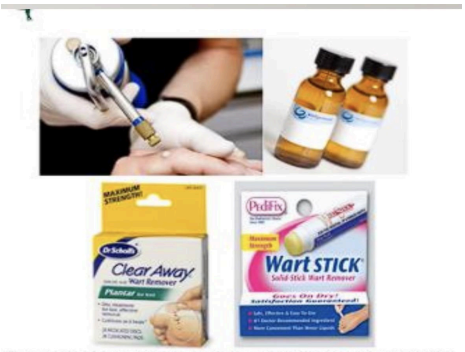


Figure 8. **Treatment for warts**. Liquid nitrogen (upper left), Podophyllin (upper right), salicylic acid (bottom)

Prophylaxis

- It's better for females to be injected with vaccines

Gardasil

- Offers protection against 4 genotypes
- Better since it targets 4 genotypes: 6, 11, 16 & 18
- Manufactured by Merck company
- Approved by DFA in 2006 for females aged 9-26 yrs. Old
- Approved in 2009 for in males since there's 6 and 11

Cervarix

- Offers protection against 2 most important oncogenic HPV (16 & 18)
- Manufactured by GSA or GalactoSmithKline
- In females, the recommended age is between 11-13 years old and until 19 years old
- For males, there's no specific age group



Infection Control

- Barrier methods during sexual intercourse
- Warts should be covered esp. during swimmin
- Can be covered with tape (The skin tends to soften if wet; if the lesion softens, since it's only the epidermis, the cover can be removed, making it more Infectious)
- No sharing of towels or walking barefoot in public areas
- Avoid the sharing of personal items
- Cesarian section
- Circumcision

TABLE 65-19 Polyomaviruses

Family	*Polyomaviridae*
Common name	Polyomavirus
Virus	Polyomavirus (BK virus [BKV] and JC virus [JCV] infect humans)
Characteristics	Double-stranded deoxyribonucleic acid (DNA) genome; icosahedral capsid, no envelope; includes BK and JC polyomaviruses
Transmission	Probably direct contact with infected respiratory secretions; both viruses are ubiquitous in humans.
Site of latency	Kidney
Disease	Mild or asymptomatic primary infection; virus remains dormant in kidneys; reactivation in immunocompromised patients causes hemorrhagic cystitis (BKV) or progressive multifocal leuko-encephalopathy (JCV).
Detection	JCV by polymerase chain reaction (PCR) (cerebrospinal fluid) or electron microscopy (EM) (brain tissue); BKV by PCR or cytology (urine)
Treatment	Supportive; decrease immune suppression
Prevention	Avoid contact with virus; prevention of acquisition of virus unlikely.

- Virion: Icosahedral, 45 nm
- Composition: protein (90%), dsDNA (10%)
- Genome: dsDNA
- Envelope: None
- **Replication**: Nucleus

Outstanding characteristics:

- Stimulate cell DNA synthesis
- Viral oncoproteins interact with cellular tumor suppressor proteins

Important model tumor viruses

- Human viruses can cause human neurologic and renal disease
- May cause human cancer
- Simian Virus 40 - from monkeys & human
- **BK virus-** human
- JC virus(human polyomavirus 2)- human
- **Murine polyoma virus -** mice

Pathogenesis

- Widely distributed in human population
- 70-80% specific Ab in adult sera
- Usually happens during early childhood
- Persist in kidney and lymphoid organs after primary infection

Reactivation in:

- Renal transplantation o Pregnancy o Increasing age
- ICP
- Asymptomatic viral reactivation & viral shedding in urine
- **Primary infection:** asymptomatic
- **Latent infection:** symptomatic

Polyomavirus hominis 1

- Common infection of childhood but specific disease has not been associated with the virus
- Infections are subclinical
- **Urine** - cystitis
- Evidence of infection has been demonstrated in children with acute respiratory illness
- Reactivates in immunosuppressed patients

- Renal transplant patients
- Urethral strictures
- BM transplant patients
- Hemorrhagic cystitis
- Parvo sheds respiratory tract
- Papilloma sheds from the skin since it's localized there
- Polyomavirus hominis 1 sheds in the urine

Treatment

- Reducing immunosuppression
- **Cidofovir** - Only antiviral agent that may be effective
- Treatment is usually given to immunocompromised patients
- **Viral infection:** treatment will be via the immune system
- **Immunocompromised patients:** needs to be helped with anti-viral treatment/agent

Laboratory Diagnosis

- PCR
- Specimen is URINE

Polyomavirus hominis 2

- AIDS pateints are greatly affected
- It affects the CNS; therefore, the specimen for PCR is the CSF or brain biopsy
- Demyelination and loss of oligodendrocytes can occur
- Antibody to the virus is highly prevalent in the general population
- Not clear what primary illness the virus cause
- Immunosuppressed patients due to T -cell dysfunction
- Virus reactivates to cause PML (Progressive multifocal leukoencephalopathy)

- Change in intellect, affection of motor functions or sensory loss
- Lead to death within a year
- Prior to antiretroviral therapy, PML develops in 2-4% of AIDS patients
- No effective treatment for PML
-

Simian virus 40

- Monkey virus
    - PML-like infection
- Highly tumorigenic: causative of tumor transformation
- Not cause human infection
- Infected polio vaccines between 1955-1963
    - For polio vaccines, monkeys are used as a host for antibody reproduction for the vaccine; monkeys are used for the manufacturing of the polio vaccine

HERPESVIRIDAE

Different clinical manifestations

1. Herpes simplex virus (HSV)

- 2 serotypes: 1 and 2*

2. Varicella-zoster virus (VZV)

- Only 21 serotype. Causes chickenpox*

3. Cytomegalovirus (CMV)

- Infects immunocompromised patients*

4. Epstein-Barr virus (EBV)

- IM (infectious mononucleosis)*

5. Human herpesvirus 6 (HHV 6)

- Roseola infantum, Exanthema infantum* -

6. Human herpesvirus 8 (HHV8)

- Associated with AIDS patients and developing Kaposis sarcoma.

IMPORTANT PROPERTIES OF HERPESVIRUSES (from Jawetz)	
Virion	Spherical, 150-200 nm in diameter
Genome	Double-stranded DNA, linear, 125-240 kbp, reiterated sequences
Proteins	More than 35 proteins in the virion
Envelope	Lipoprotein; contains viral glycoproteins, and Fc receptors
Core	Icosahedral core
Replication	Nucleus, buds from the nuclear membrane; Forms intranuclear inclusions
Outstanding characteristics	1. Encodes many enzymes 2. Establish latent infections 3. Persist indefinitely in infected hosts 4.Frequently reactivated in immunosuppressed hosts 5. Some may cause cancer

Table 1. Important properties of Herpesviruses

TABLE 65-12 Herpesviruses

Family	*Herpesviridae*
Common name	Herpesvirus (HSV)
Characteristics	Double-stranded deoxyribonucleic acid (DNA) genome; icosahedral capsid with envelope; at least eight human herpes viruses known: HSV-1, HSV-2, varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus (HHV)-6, HHV-7, and HHV-8
Virus	HSV-1 and HSV-2
Transmission	Direct contact with infected secretions
Site of latency	Sensory nerve ganglia
Disease	Predominant virus in parentheses: gingivostomatitis (HSV-1), pharyngitis (HSV-1), herpes labialis (HSV-1), genital infection (HSV-2), conjunctivitis (HSV-1), keratitis (HSV-1), herpetic whitlow (HSV-1 and HSV-2), encephalitis (HSV-1 in adults), disseminated disease (HSV-1 or HSV-2 in neonates)
Detection	Cell culture (HDF, others), EIA, FA stain, IH stain, PCR
Treatment	Acyclovir, valacyclovir, famciclovir
Prevention	Avoid contact.
Virus	VZV
Transmission	Close personal contact, especially respiratory
Site of latency	Dorsal root ganglia
Disease	Chicken pox (varicella), shingles (zoster)
Detection	FA stain, cell culture (HDF), shell vial culture, PCR
Treatment	Acyclovir and famciclovir
Prevention	Vaccine
Virus	EBV
Transmission	Close contact with infected saliva
Site of latency	B lymphocytes
Disease	Infectious mononucleosis, progressive lymphoreticular disease, oral hairy leukoplakia in patients with HIV
Detection	Serology, PCR
Oncogenic	Burkitt lymphoma, nasopharyngeal carcinoma
Treatment	Supportive
Prevention	Avoid contact.
Virus	CMV
Transmission	Close contact with infected secretions, blood transfusions (WBCs), organ transplants, transplacental
Site of latency	WBCs, endothelial cells, cells in a variety of organs
Disease	Asymptomatic infection, congenital disease of newborn, symptomatic disease of immunocompromised host, heterophile-negative infectious mononucleosis
Diagnosis	Cell culture (HDF), shell vial culture, CMV antigenemia, FA stain, PCR
Treatment	Supportive; decrease immune suppression; ganciclovir and foscarnet
Prevention	Use CMV antibody–negative blood and tissue for transfusion and transplantation, respectively.
Virus	HHV-6 and HHV-7
Transmission	Most likely close contact via respiratory route; almost all children infected by age 2-3 years
Site of latency	T lymphocytes (CD4 cells)
Disease	Roseola (exanthem subitum), fever, malaise, rash, leukopenia, and interstitial pneumonitis in organ transplant recipients
Detection	Detection of virus in peripheral blood specimens by PCR, cell culture using lymphocyte lines
Treatment	Susceptible to ganciclovir and foscarnet
Prevention	None practical
Virus	HHV-8
Transmission	Not known; much less widely disseminated than other herpes viruses
Site of latency	Viral genome found in Kaposi tumor cells, endothelial cells, and tumor-infiltrating leukocytes
Disease	Kaposi sarcoma
Detection	PCR or *in situ* by hybridization
Treatment	None known
Prevention	Avoid contact with virus.

EIA, Enzyme immunoassay; *FA*, fluorescent antibody; *HDF*, human diploid fibroblast; *HIV*, human immunodeficiency virus; *IH*, iron hematoxylin; *PCR*, polymerase chain reaction; *WBCs*, white blood cells.

Incubation period

- 2-12 days

Infectious period

- From onset of symptoms until the lesions are fully crusted or resolved
- Usually, for HSV, from the onset of the manifestation until the lesion is resolved.

Mode of Transmission

- Sexual contact
- Skin, genital or eye contact with vesicle fluid from HSV skin lesion

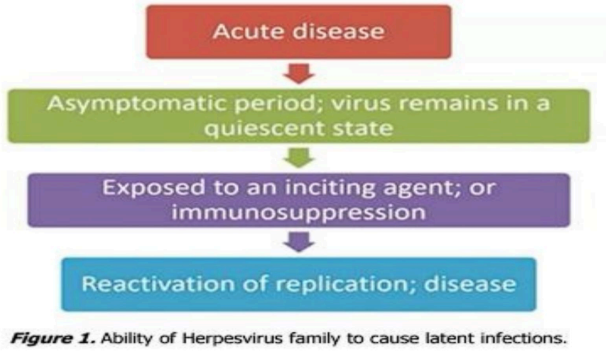


Figure 1. Ability of Herpesvirus family to cause latent infections.

THREE CATEGORIES

A. Alpha Herpesvirus

- HSV 1 and 2
- VZV

- Infect epithelial cells like EC on the skin that is why in these viral infections, there is skin rash or lesions

- Latent in neurons

B. Beta Herpesvirus

- CMV
- HHV 6

- Variety of tissues: site of latency or the type of cells most often infected varies

C. Gamma Herpesvirus

- EBV
- HHV 8

- Lymphoid cells: site of latency

8 HUMAN HERPES THAT INFECT DIFFERENT ORGANS

- Skin
- Lymphatic system
- CNS

1. HSV 1
2. HSV 2
3. VZV/HHV3
4. EBV
5. HHV 6
6. CMV/HHV5
7. HSV
8. CMV

- Neuroinvasive, neurovirulent

- Can also be neuroinvasive

Diseases

1. HSV- 1

- Acute gingivostomatitis
- Recurrent herpes labialis (cold sores)
- Keratoconjunctivitis (keratitis)
- Encephalitis

2. HSV- 2

- Herpes genitalis
- Neonatal herpes

- Aseptic meningitis

a. Simplex virus genera



Figure 12. Gingivostomatitis affecting a 3-year-old. Lesions from HSV - 1 are seen on the face, mouth, lip, and chin.



Species:

- HSV 1
- HSV 2

1. Herpes simplex virus (HSV-1)

- Initial infection occur in early childhood
- Portal of entry: oral mucosa
- Gingivostomatitis
- Latency site- trigeminal ganglion

After infecting the oral mucosa, it goes back to the trigeminal ganglion, and when your immune system weakens, it goes again to the oral mucosa and causes sores/blisters.

Again and again

- Trigeminal ganglion – aka Gasseri ganglion
    - Common cause of viral encephalitis

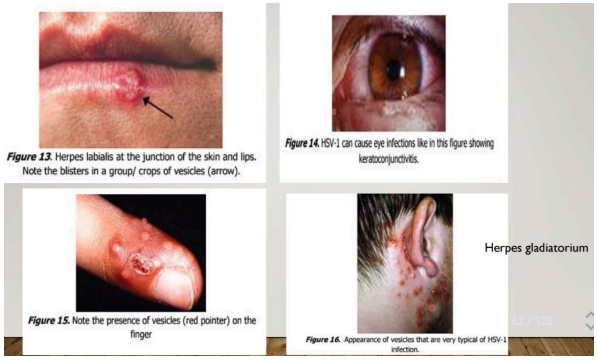


Figure 13. Herpes labialis at the junction of the skin and lips. Note the blisters in a group/ crops of vesicles (arrow).

Figure 14. HSV-1 can cause eye infections like in this figure showing keratoconjunctivitis.

Figure 15. Note the presence of vesicles (red pointer) on the finger

Figure 16. Appearance of vesicles that are very typical of HSV-1 infection.

Herpes gladiatorium



Figure 19. In this case, the vesicles are seen on the scalp of the newborn. Acquired by passing through infected birth canal.

Figure 20. Example of severe infection

Figure 17. Note the presence of vesicles that are present near the glans penis (can also be found on the shaft) and on the vulva . These vesicles are found in groups or crops.

HSV-1 or HSV-2 is a common cause of **<u>erythema multiforme</u>**



Figure 5. Erythema multiforme

- Produces a characteristic skin rash
- The rash of erythema multiforme appears as a central red area surrounded by a ring of normal skin outside of which is a red ring
- This lesion is characterized as a target or **bull's eye lesion**
- The lesions are typically macular or papular; it occurs symmetrically on the trunks, hands and feet
- The rash is thought to be an immune-mediated reaction to the presence of HSV antigens

1. Herpes simplex virus (HSV-2)



Normally affects the urogenital area

- Genital herpes

- Can be contracted despite the an existing

HSV type I infection

- Neurological infections are rare and more

benign - Latency site: lumbosacral ganglia or peripheral tissues

- Genital herpes
- Neonatal herpes
- Vertical transmission – when the delivery is through the birth canal.
- Aka **herpes neonatorum**

Transmission

How is HSV transmitted?

HSV-1

- Primarily in saliva
- Infections mainly on the face

HSV-2

- Sexual contact
- Lesions in genital area

Epidemiology

- HSV-1: Most primary infections in childhood
- HSV-2: Age of sexual activity

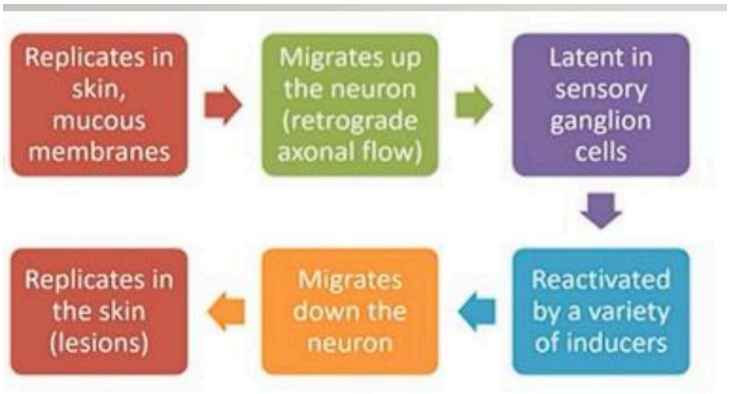


Figure 6. Pathogenesis of HSV infection in general

HSV-I becomes latent in the sensory ganglion cells

- HSV-I: becomes latent in the trigeminal ganglia
- HSV-2: becomes latent in the sacral ganglia

The virus can be reactivated by a variety of inducers

- Sunlight
- Hormonal changes
- Trauma
- StressFever

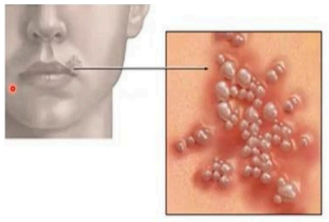


Figure 7. Vesicles. Elevated lesions containing fluid



Figure 8. Multinucleated giant cells

Both reinfection and reactivation occur in the presence of circulating IgG

- Even if an individual has antibodies, there can still be reinfection and the phenomenon of reactivation
- Immunity is incomplete

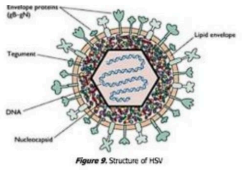


Figure 9. Structure of HSV

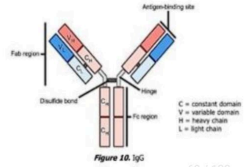


Figure 10. IgG

	HSV-1	HSV-2
Location of lesions	Generally, **above** the waist	Generally, **below** the waist
Diseases	1. Acute gingivostomatitis (mouth or gum lesions) 2. Recurrent herpes labialis (cold sores/ singaw) 3. Keratoconjunctivitis (keratitis) 4. Encephalitis	1. Herpes genitalis (Genital herpes) 2. Neonatal Herpes by passing through the infected birth canal 3. Aseptic meningitis (Etiologic agent cannot be grown in routine culture media)
Transmission	Primarily in the saliva (Infections mainly on the face)	Sexual contact (Lesions on the genital area)
Age group affected	Most primary infection in childhood	Age of sexual activity
Site of latency	Trigeminal ganglia	Sacral ganglia

Table 2. Summary of Comparison between HSV-1 and HSV-2

Laboratory diagnosis:HSV-1, HSV-2

1. Scraping from the base of the vesicles stained with Giemsa

- TZANCK smear = (+) multinucleated giant cells with cowdry inclusion: cytopathic effect (CPE)
- Scrape on a slide, stain it with giemsa.*

2. IFT

- Immunofluorescence test*
- Detection of antibody*

3. Cell Culture - CPE (multinucleated giant cells with cowdry inclusion bodies) occur in 3 days then ID by IFT

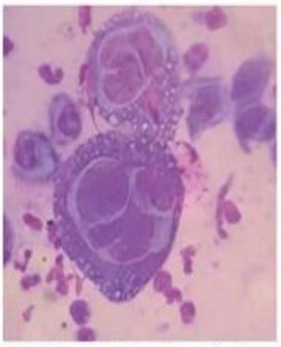
- 1 and 2: CAM (chlorioallantoic membrane/chorionic cell membrane)*

4. PCR

- Molecular diagnosis*
- Specimen: blister content, tissues*

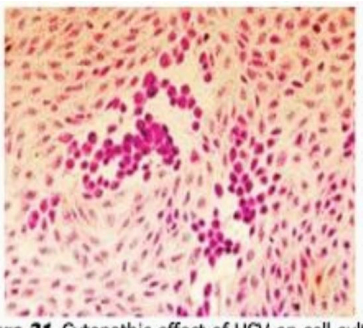
5. EM

- Specimen: blister content*
- Directly visualize organism under EM*


Figure 4. Multinucleated cell seen in Tzanck smear

LABORATORY DIAGNOSIS OF HSV INFECTIONS

A. Cell Culture


Figure 21. Cytopathic effect of HSV on cell culture.

B. Antibody Neutralization/ Fluorescent Antibody Test

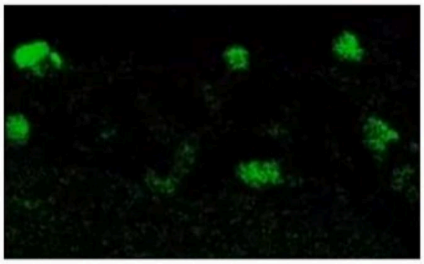


Figure 22. Positive result of Fluorescent antibody test.

- Can also be identified by antibody neutralization or fluorescent antibody test.

TREATMENT

A.Acyclovir

- Acycloguanosine, Zovirax
- Used for the treatment of severe infections like encephalitis and disseminated disease wherein the drug is given intravenously.
- Useful in the treatment of neonatal infections
- Useful in the treatment of primary and recurrent genital infections and in this case, the drug can be administered orally rather than IV
- It shortens the duration of the lesions and reduces the extent of shedding of the virus but does not cure the latent state.
- Treatment of genital herpes with acyclovir in a pregnant woman appears to be safe for both mother and developing fetus

B. Trifluorothymidine – treatment for keratitis (topical)

b. Varicellovirus - genera

- Varicella Zoster Virus specie

Varicella-zoster Virus (VZV)

Mode of Transmission

- Airborne route
- From vesicles of the skin
- Initial and second period of viremia
- Incubation period ▪ 14-21 days
- Infectious Period ▪ 2 days before the onset of symptoms (fever and lesion) until 5 days after the rash or all of the skin lesion are fully crusted
- Generalized vesicular skin

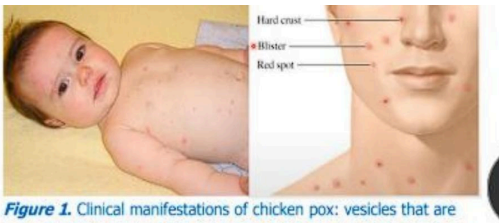


Figure 1. Clinical manifestations of chicken pox: vesicles that are present on the trunk of the child. **Typical lesion**, just like HSV, a **blister or vesicle** that start as **erythematous or red spot** and develops into blister and eventually into a dry lesion called **hard crust**



Figure 2. Distribution of vesicles: they are not arranged in crops or in groups unlike in HSV infection. You see them scattered or occurring individually and in different stages



Varicella "Chickenpox"	Zoster "Shingles"
Primary infection	Reactivation of latent infection
Children	adults
Begins at the trunk and spread to the face and extremities; centrifugal pattern	Follows dermatomal pattern

Table 1. Diseases caused by VZV

TRANSMISSION : RESPIRATORY DROPLETS

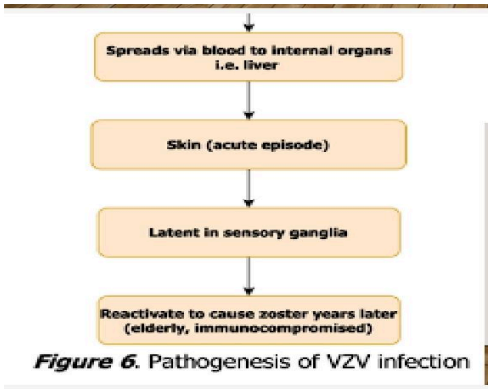


Figure 6. Pathogenesis of VZV infection

Steps in Pathogenesis

1. Varicella-zoster virus is inhaled; infects mucosal cells in nose and throat

2. The virus infects nearby lymph nodes, replicates, and enters the bloodstream (primary viremia)

3. Infection of other body cells occurs, with replication in liver and spleen, resulting in secondary viremia

4. The virus causes successive crops of skin lesions, which evolve into blisters and crusts

5. Immune system eliminates the infection except for some virions that establish latent infections inside nerve cells

6. If immunity wanes with age or other reason, the virus persisting in the nerve ganglia can infect the skin, causing herpes zoster

7. Transmission to others occurs from respiratory secretions and skin

   a. In latently infected cells in the DORSAL ROOT GANGLIA:

      i. VZV DNA located in the nucleus

      ii. VZV DNA is not integrated into cellular DNA

   b. If immunity is reduced, virus is reactivated and causes vesicular lesions and nerve pain of ZOSTER

People at risk to develop VZV: Pregnant women and unborn babies*

- Pregnant women: 1st 20 weeks of pregnancy
- Babies: 1 week after birth.

- No subtypes

- Portal of entry: nasopharyngeal space and conjunctiva

- Initial infection: chickenpox/ varicella

- Confers an effective immunity

  - can reactivate as shingles (latency infection).

Shingles: more severe than chickenpox.*

- Latency site: spinal ganglia or dorsal root of ganglia

- Zoster as reactivation develop

- Reactivation is induced by external and internal influences

Laboratory Diagnosis

- Cell culture - gold standard for diagnosis of viral infection
- The virus causes CPE in cell culture



Figure 8. VZV fluoresce against a dark background

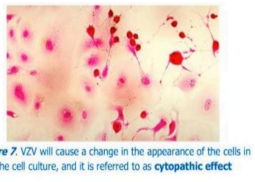


Figure 7. VZV will cause a change in the appearance of the cells in the cell culture, and it is referred to as **cytopathic effect**



Figure 9. Live attenuated varicella virus vaccine

VARIVAX	ZOSTAVAX
Varicella protection	Prevent Zoster Virus
Children 1-12 y/o	>60 y/o
2 dosages	1 dose
	Contains 14x more virus than varicella vaccine
Contraindication: Immunocompromised, pregnant	
Both contain live, attenuated VZV	
Active immunity	

Table 2. Varivax and Zoster Vax

c. Cytomegalovirus (genera)

- Cytomegalovirus (CMV) – specie

CMV:

- Primary infection: asymptomatic*
- Latent infection: symptomatic*

Transmission: Transplacental, oral, sexual, and by blood and tissue transplant

#1 common cause of Congenital infection in the USA

- Transplacental: crosses the placenta during conception.
- can also be transmitted through sexual intercourse, blood transfusion, or tissue/organ transplant.

- Infection: Mononucleosis like but heterophile Ab(-)

- "**Owl eye"** inclusion: CPE

- Culture: HDF: human diploid fibroblast cell (method of choice)

PAP(Pap's smear)/Giemsa (TZANCK smear) – urine/tissue*

- Owl eye inclusion

- CMV was previously known as HHV-5*



Incubation period

- 3-6 weeks - Infectious period
- Immunocompetent
  - Prolonged after primary infection

Immunocompromised

- Experienced reactivation
- Weeks or months

- Initial infection: inapparent in most persons
- Latency site: mononuclear cell

- Reactivation also occurs asymptomatic but symptoms may develop that are gen. mild

Transmission> Transmitted via body fluids

- The virus is found in human body fluids:
  - Blood Saliva Semen Cervical mucus
  - Breast milk Urine

It can also occur via

- Across the placenta – why it can lead to congenital anomalies
- By organ transplantation

PATHOGENESIS

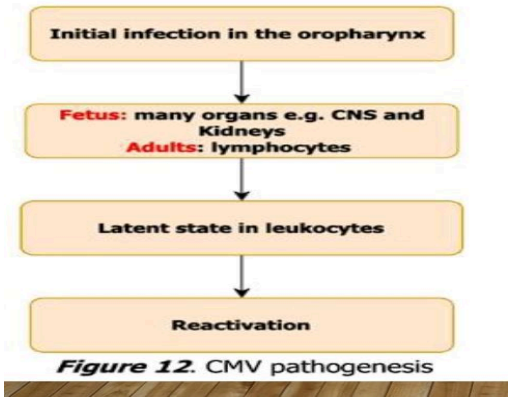


Figure 12. CMV pathogenesis

Disseminated infection in immunocompromised patients can result from:

- Primary infection
- Reactivation of latent infection

Laboratory Diagnosis

The virus causes CPE in cell culture> Viral/ Cell Culture (**GOLD STANDARD**)

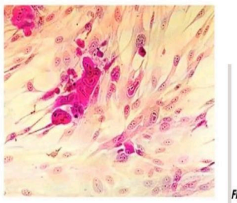


Figure 13. CPE in cell culture of CMV

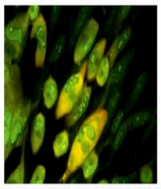


Figure 14. Fluorescent Antibody Test of CMV. **Positive Resul** structure fluoresce against a dark background

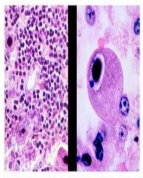


Figure 15. Intranuclear Inclusion bodies of CMV. *Left: LPO, Right:* HPO (very large intranuclear inclusion body, that's why it is called **Cytomegalic inclusion body disease**)

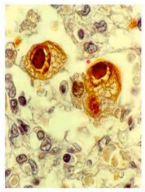


Figure 16. Very large inclusion bodies, cyto"**MEGA**"= large cell



Figure 17. Microwell ELISA (Cytomegalovirus IgG)

Treatment

- **Ganciclovir** is beneficial in treating pneumonia and retinitis > Administered intravenously

SUMMARY OF DRUGS	
Ganciclovir (*Cytovene*)	Moderately effective in the treatment of **CMV retinitis** and **pneumonia** in patients with **AIDS**
Valganciclovir	-Can be taken **orally** -Also effective against **CMV retinitis**
Foscarnet (*Foscavir*)	Also effective but causes **more side effects**
Cidofovir (*Vistide*)	Also useful in the treatment of **CMV retinitis**
Fomivirsen (*Vitravene*)	- An antisense DNA approved for the **intraocular treatment of CMV retinitis** - The first and, at present, the **only antisense molecule** to be **approved** for the treatment of human disease

Table 3. Summary of drugs. *from 2022 trans

EPSTEIN-BARR VIRUS (EBV)

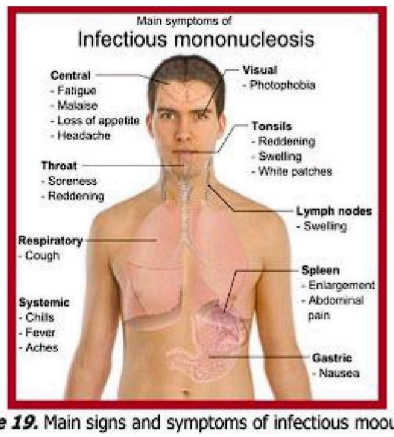


Figure 19. Main signs and symptoms of infectious mooucleosis

Disease

- EBV causes Infectious Mononucleosis

Signs and symptoms:

- Central: fatigue, malaise, loss of appetite, headache
- Visual: photophobia
- Tonsils: reddening, swelling, white patches
- Throat: soreness, reddeningLymph

- Nodes: SwellingRespiratory: cough
- Spleen: Enlargement, abdominal pain
- Gastric: nausea
- Systemic: chills, fever, a
- EBV is associated with Burkitt's lymphoma in East African children (actually even in adults)



Figure 20. Patient with Burkitt's lymphoma

Nasopharyngeal Carcinoma

- Other malignancy associated with
- EBVEBV also causes **HAIRY LEUKOPLAKIA**
- The replicative cycle is similar to that of HSV
- EBV enters B lymphocytes at the site of the receptor for the C3 component of complement
- Humans are the natural hosts.

Primary Infection

- EBV infects mainly lymphoid cells, primarily B lymphocytes.
- EBV also infects the epithelial cells of the pharynx, resulting in prominent sore throat.

Latent Infection

- EBV DNA is in the nucleus and is not integrated into cellular DNA.
    - Some genes, but not all, are transcribed, and only a subset of those are translated into protein

Transmission

- The virus is found in the human oropharynx and in B lymphocytes.
- It is transmitted primarily by saliva>
- AKA "**KISSING DISEASE"**

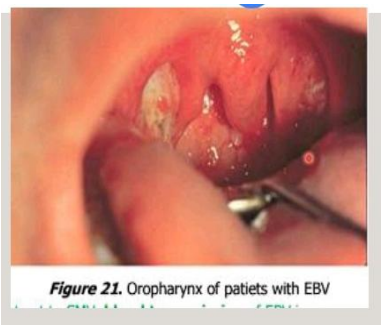


Figure 21. Oropharynx of patiets with EBV

PATHOGENESIS

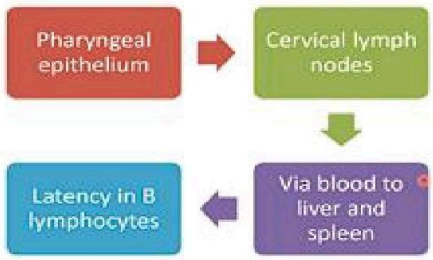


Figure 22. From the **pharyngeal epithelium** it travels to the **cervical lymph nodes**. From there, transported via **blood** and **spleen** and the site of **latency in B lymphocytes**.

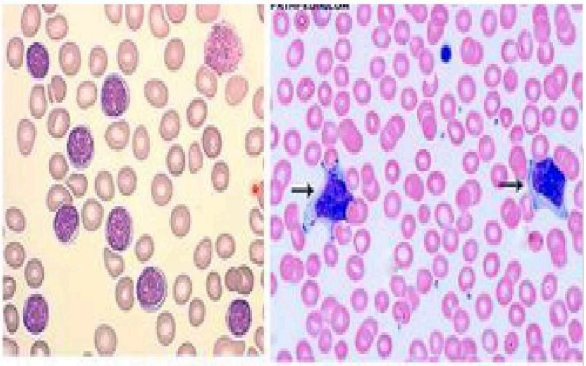


Figure 23. Left: Lymphocytosis. Right: atypical lymphocytes.

LABORATORY DIAGNOSIS

Monospot test

- Heterophil antibody is typically positive (MonospotTest)
- Heterophil antibody agglutinates sheep or horse red blood cells.
    - act on an antigens different from the antigen that stimulated the production of antibody



Figure 24. Monospot Test. One of the classic or initial tests that were used to diagnose EBV

Viral Capsid Antigen (VCA)

- A significant rise in EBV-specific antibody to viral capsid antigen (VCA) is diagnostic
- Better test and more specific compared to Monospot test



Figure 25. EBV-specific antibody tests

Treatment and Prevention

- Treatment: No effective drug is available for treatment
- Prevention: There is no drug or vaccine for prevention.

HUMAN HERPESVIRUS 8



*Figure 26. Typical skin lesions of Kaposi's Sarcoma. The image shows the **typical skin lesions** of Kaposi's Sarcoma and the lesions have a purple color due to collections of RBC*

Disease

- Human Herpesvirus 8 causes Kaposi's Sarcoma in HIV AIDS patients.

Pathogenesis

- HHV-8 causes malignant transformation by a mechanism similar to that of other DNA viruses (i.e. HPV)
- Inactivation of a tumor suppressor geneA protein encoded by HHV-8 called latency-associated nuclear antigen

- (LANA) inactivates RB and p53 tumor suppressor proteins, causing malignant transformation of endothelial cells

Transmission

- Transmission of HHV-8 occurs primarily via:
  - Sex
  - Saliva
- Can also be transmitted in transplanted organs
  - Kidneys
  - Appears to be the cause of transplantation- associated KS
- DNA of HHV-8 is found in the cells of transplantation-associated KS but NOT in the cells of other transplantation- associated cancer

Laboratory Diagnosis

- The diagnosis is made by pathologic examination of the lesion.
- Laboratory diagnosis of KS is often made by biopsy of the skin lesions.
- HHV-8 DNA and RNA are present in most spindle cells and can be detected by PCR assay.
- Virus is NOT grown in culture.

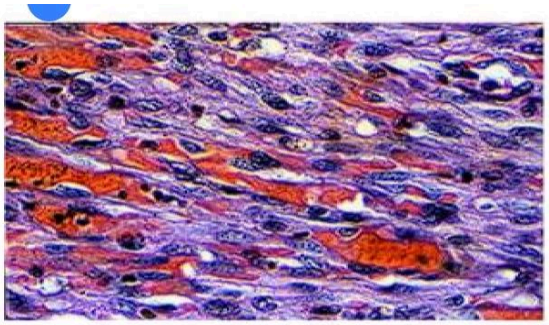


Figure 27. Pathologic Finding of HH8

<u>Treatment</u>

- The type of treatment depends on the:
  - Site of lesions
  - Number of lesions
- Different treatment modalities that can be used:
  - Surgical excision
  - Radiation
  - Chemotherapy
  - Immunomodulatory drugs (i.e. alpha interferon)
- Early HIV-associated KS: highly active antiretroviral drugs (HAART) can be effective treatment
- Anti-herpesvirus drugs (acyclovir, foscarnet, cidofovir): NOT effective
- HAART: can also prevent KS as evidenced by a decreased incidence of KS in HIV-infected patients treated with HAART
- NO vaccine against HHV-8
-

POXVIRUS

- Poxvirus family includes three viruses of medical importance: smallpox virus, vaccinia virus, and molluscum contagiosum virus.
- Poxviruses are the largest and most complex of viruses infecting humans
- They are similar morphologically and share a common nucleoprotein antigen
- Infections with most poxviruses are characterized by a rash, although lesions induced by some members of the family are markedly proliferative

SMALLPOX VIRUS

- WHO: has been eradicated
- An example for one of the triumphs of vaccination
- Also called Variola virus

A. Disease

- Agent of smallpox
- Only human disease that has been eradicated from the face of the Earth.
- Eradication was achieved by the widespread use of the smallpox vaccine.
- There is concern regarding the use of smallpox virus as an agent of bioterrorism.

Transmission

- Transmitted via respiratory aerosol or by direct contact with virus either in the skin lesions or on fomites such as bedding.

Pathogenesis

- Smallpox begins when the virus infects the upper respiratory tract and local lymph nodes and then enters the blood (primary viremia).
- Internal organs are infected; then the virus re-enters the blood(secondary viremia) and spreads to the skin.
- These events occur during the incubation period, when the patient is still well.
- The rash is the result of virus replication in the skin, followed by damage caused by cytotoxic T cells attacking virus-infected cells.
- Immunity following smallpox disease is lifelong
- Immunity following vaccination lasts about 10 years.

Laboratory Diagnosis

- In the past when the disease occurred, the diagnosis was made either by growing the virus in cell culture or chick embryos or by detecting viral antigens in vesicular fluid by immunofluorescence.

MOLLUSCUM CONTAGIOSUM

Disease:

- Molluscum contagiosum virus causes molluscum contagiosum



- So initially they look like Herpes virus infections but the crater in the middle will point towards Molluscum contagiosum
- It is transmitted by direct contact.
- The diagnosis is made clinically rather than laboratory means

Treatment and Prevention

- There is no established antiviral therapy and no vaccine.
- Cidofovir may be useful in the treatment of extensive lesions in immunocompromised patients.



Figure 30. Cidofovir

PAPILOMAVIRUS

TABLE 65-14 Papillomaviruses

Family	*Papovaviridae*
Common name	Papillomavirus
Characteristics	Double-stranded DNA genome; icosahedral capsid, no envelope; includes papilloma viruses
Virus	Human papilloma virus (HPV)
Characteristics	Contains more than 200 deoxyribonucleic acid (DNA) types
Transmission	Direct contact, sexual contact for genital warts
Site of latency	Epithelial tissue
Disease	Skin and genital warts, benign head and neck tumors, anogenital warts
Diagnosis	Cytology, DNA probes
Oncogenic	Cervical and penile cancer (especially HPV types 16 and 18)
Treatment	Spontaneous disappearance the rule; surgical or chemical removal may be necessary
Prevention	Avoid contact with infected tissue, vaccination.

Human Papillomavirus

- Small, non-enveloped, circular, double stranded DNA viruses
- Abundant in nature
- Causes infections in humans, dogs, cattle, monkeys and many other species
- 200 genotypes (based on viral sequences) w/ ~80 are well characterized
- Much attention focused on more than 30 sexually transmitted genotypes and its role in the pathogenesis of cancer (cervical cancer)
- These various genotypes have different cellular tropisms which helps it establish different variations in its clinical presentation
- Can cause warts

TABLE 43-6 Important Properties of Papillomaviruses[a]

Virion: Icosahedral, 55 nm in diameter
Composition: DNA (10%), protein (90%)
Genome: Double-stranded DNA, circular, 8 kbp, MW 5 million
Proteins: Two structural proteins; cellular histones condense DNA in virion
Envelope: None
Replication: Nucleus
Outstanding characteristics: Stimulate cell DNA synthesis Restricted host range and tissue tropism Significant cause of human cancer, especially cervical cancer Viral oncoproteins interact with cellular tumor suppressor proteins

[a]Formerly classified in Papovaviridae family.
MW, molecular weight.

Figure 2. Important Properties of Papillomaviruses

Human Papillomavirus (HPVs)

- Human warts
- Tropism for either cutaneous or mucosal tissues

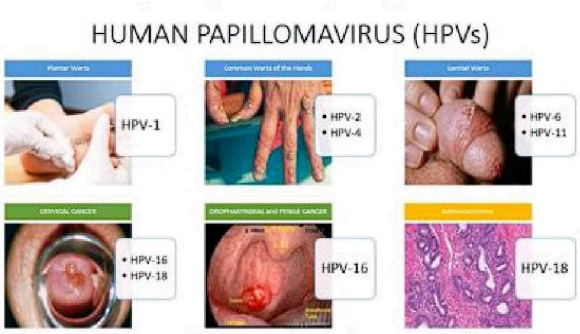


Figure 6. Common Human Papillomavirus seen in Clinical Practice

15-20 types *of HPVs that can cause virtually all cases of cervical cancer, but among those,* ***HPV 16 and 18 which causes >60% of cases***

- **HPV 16** – subset of cancers of the oropharynx and penile cancers
- **HPV 18** – associated with adenocarcinoma of the cervix
- **HPV 1** – benign types with plantar warts
- **HPV 4 and 2** – common wart of the hands
- **HPV 6 and 11** – genital warts
- **Benign types** – HPV 1, 2, 4, 6, 11
- **Potentially malignant** – HPV 16 and 18

TABLE 43-7 Examples of Association of Human Papillomaviruses With Clinical Lesions

Human Papillomavirus Type*	Clinical Lesion	Suspected Oncogenic Potential
1	Plantar warts	Benign
2, 4, 27, 57	Common skin warts	Benign
3, 10, 28, 49, 60, 76, 78	Cutaneous lesions	Low
5, 8, 9, 12, 17, 20, 36, 47	Epidermodysplasia verruciformis	Mostly benign, but some progress to malignancy
6, 11, 40, 42–44, 54, 61, 70, 72, 81	Anogenital condylomas; laryngeal papillomas; dysplasias and intraepithelial neoplasias (mucosal sites)	Low
7	Hand warts of butchers	Low
16, 18, 30, 31, 33, 35, 39, 45, 51–53, 56, 58, 59, 66, 68, 73, 82	High-grade dysplasias and carcinomas of genital mucosa; laryngeal and esophageal carcinomas	High correlation with genital and oral carcinomas, especially cervical cancer

*Not all papillomavirus types are listed.

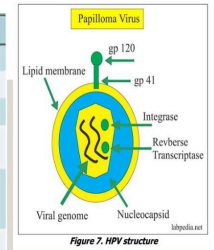


Figure 7. HPV structure

Laboratory Diagnosis

- Cannot be cultivated in cell culture due to difficulty of procedure
- Disadvantage: No production of type specific antigens and corresponding anti-sera
-

Histopathology or Cytopathology

- **Cytopathology** – can be used as initial screening test for patients for cervical cancer
- Aims to detect pre-cancerous cellular changes in the morphology
- Allows viewing of certain lesions which necessitates removal prior to cancer development

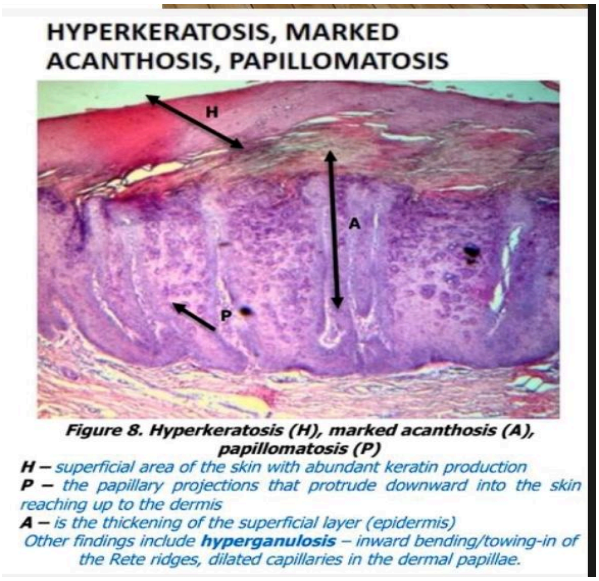


Figure 8. Hyperkeratosis (H), marked acanthosis (A), papillomatosis (P)

H *– superficial area of the skin with abundant keratin production*
P *– the papillary projections that protrude downward into the skin reaching up to the dermis*
A *– is the thickening of the superficial layer (epidermis)*
Other findings include ***hyperganulosis*** *– inward bending/towing-in of the Rete ridges, dilated capillaries in the dermal papillae.*



Figure 9. Koilocytes (arrows) *– typical for HPV; vacuolated superficial keratinocytes that have a pyknotic raisin-like nucleus surrounded by an area of clearing*

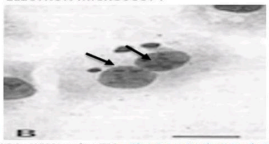


Figure 10A. HPV under EM *with intra-cytoplasmic electron-dense inclusions within the cell (arrows)*



Figure 11A. Papanicolaou (PAP) Smear *showing collection of epithelial cells with increased size of the cells with abundant well-defined cytoplasm and nucleus being slightly enlarged and can reach 3 or more times greater in size*

2. DNA Probe Assays

- Done when cytopathology is not enough to diagnose
- Identification of specific genotypes which are HPV 16 and 18 including other high-risk HPV types

- Enhance detection sensitivity and specificity of pre-cancerous lesions
- Commonly done with DNA hybridization and/or PCR method for specific identification of high-risk genotypes
- SPECIMEN: Single, liquid based cytology sample or liquid prep samples of Pap smear

POLYOMAVIRUSES

TABLE 43-5 Important Properties of Polyomaviruses*

Virion: Icosahedral, 45 nm in diameter
Composition: DNA (10%), protein (90%)
Genome: Double-stranded DNA, circular, 5 kbp, MW 3 million
Proteins: Three structural proteins; cellular histones condense DNA in virion
Envelope: None
Replication: Nucleus
Outstanding characteristics: Stimulate cell DNA synthesis Viral oncoproteins interact with cellular tumor suppressor proteins Important model tumor viruses Human viruses can cause human neurologic and renal disease May cause human cancer

*Formerly classified in Papovaviridae family.
MW, molecular weight.

Figure 14. Important properties of Polyomaviruses

A. JC Virus

- John Cunningham virus
- Disease in the CNS
- Progressive multifocal leukoencephalopathy

B. BK Virus

- BK is the initials of the first renal transplant patient who ever have infection with the virus
- Hemorrhagic cystitis

Laboratory Diagnosis

1. JC Virus

- CSF Samples – PCR
  - faster (ideal), enough for diagnosis
- Brain tissue – Histopathology, Electron microscopy, IHC

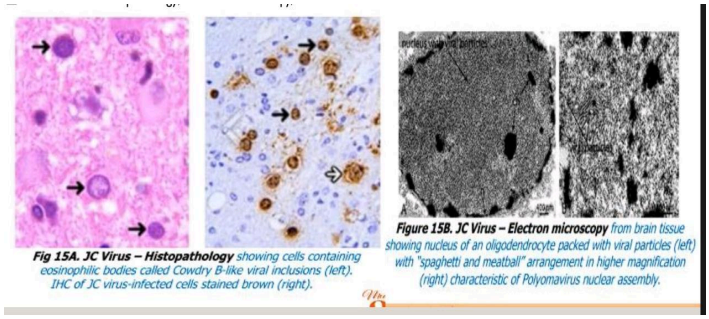


Fig 15A. JC Virus – Histopathology *showing cells containing eosinophilic bodies called Cowdry B-like viral inclusions (left). IHC of JC virus-infected cells stained brown (right).*

Figure 15B. JC Virus – Electron microscopy *from brain tissue showing nucleus of an oligodendrocyte packed with viral particles (left) with "spaghetti and meatball" arrangement in higher magnification (right) characteristic of Polyomavirus nuclear assembly.*

2. BK Virus

- Urine – PCR or cytologic examination which are only available in reference laboratories since BK virus rarely causes hemorrhagic cystitis

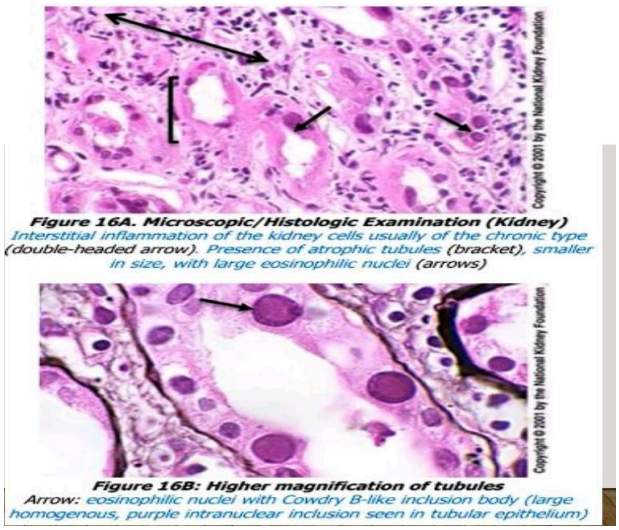


Figure 16A. Microscopic/Histologic Examination (Kidney)
Interstitial inflammation of the kidney cells usually of the chronic type (double-headed arrow). Presence of atrophic tubules (bracket), smaller in size, with large eosinophilic nuclei (arrows)

Figure 16B: Higher magnification of tubules
Arrow: eosinophilic nuclei with Cowdry B-like inclusion body (large homogenous, purple intranuclear inclusion seen in tubular epithelium)

HEPADNAVIRIDAE

HEPADNAVIRUSES

A. Hepatitis B Virus

- **“hepa-”** – Hepatitis (predilection for liver tissue)
- “-dna” for the genome type
- Pleomorphic, enveloped viruses
- Circular, partially double stranded DNA
- Replicates through an RNA intermediate, reverse transcription and DNA replication
- Asymptomatic states in children
- Acute or chronic hepatitis for adults
- Significant cause of liver damage with significant mortality and morbidity
- Eventual development of hepatocellular carcinoma in predisposed individuals

TABLE 65-11 Hepadnaviruses

Family	*Hepadnaviridae*
Common name	Hepadnavirus
Virus	Hepatitis B virus (HBV)
Characteristics	Partly double-stranded deoxyribonucleic acid (DNA) genome; icosahedral capsid with envelope; virion (also called Dane particle); surface antigen originally termed *Australia antigen*
Transmission	Humans are reservoir and vector; spread by direct contact, including exchange of body secretions, recipient of contaminated blood products, percutaneous injection of virus, and perinatal exposure
Site of latency	Liver
Disease	Acute infection with resolution (90%); fulminant hepatitis, most coinfected with delta virus (1%); chronic hepatitis, persistence of hepatitis B surface antigen (HBsAg) (9%) followed by resolution (disappearance of HBsAg), asymptomatic carrier state, chronic persistent (systemic disease without progressive liver disease), or chronic active disease (progressive liver damage)
Diagnosis	Serology, viral antigen detection, and polymerase chain reaction (PCR)
Oncogenic	Liver cancer
Treatment	Antivirals and liver transplant for fulminant disease
Prevention	HBV vaccine; hepatitis B immune globulin

Figure 23. Important characteristics of Hepadnaviruses

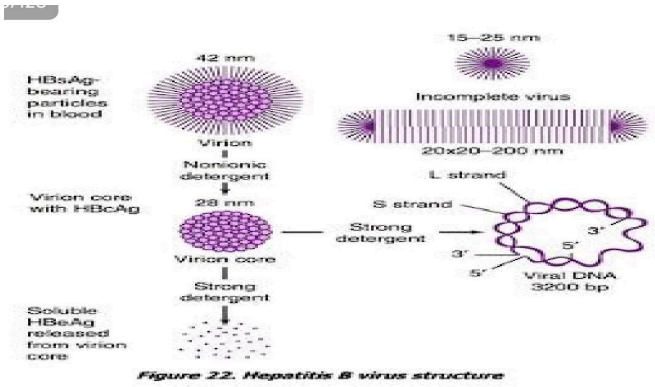


Figure 22. Hepatitis B virus structure

Laboratory Diagnosis

- Dictum for the diagnosis of Hepatitis
- Liver function tests: establishes degree of damage that the liver has undergone
  - Serum Alanine Aminotransferase (ALT)
  - Aspartate Aminotransferase (AST)
  - Bilirubin

Tissue Biopsy

1. Cell CultureNOT cultivatable in vitro

2. Histopathology and Immunohistochemistry

- Readily available in the laboratory

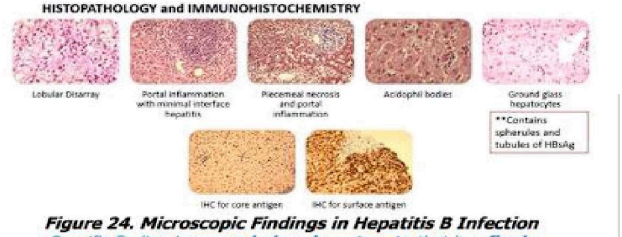


Figure 24. Microscopic Findings in Hepatitis B Infection
*Specific finding is **ground glass hepatocyte** that has **finely granular eosinophilic cytoplasm** due to presence of **spherules and tubules of HBsAg, and central ballooning degeneration.***
IHC will detect core antigen and surface antigen.
(Refer to Appendix D for enlarged image)

Electron Microscopy

- Three morphologic forms of hepatitis B surface antigen (HBsAg)

1. Spherical particles
2. Filamentous/Tubular forms
3. DANE particles

- A – Spherical particles
- B – Filamentous/Tubular forms around 200nm long, may result from overproduction of HBsAg
- C – DANE Particles (larger spherical virions, less frequently observed from clinical specimens but specific for Hepa B infection)



Figure 25. HBsAg morphologic forms under EM

<u>Immunoassays – ELISA/EIA</u>

- Identification of specific viral antigens or antibodies
- Determines the acute of chronic stat of the infection by looking at the various timelines of the antibody produced against these different antigens

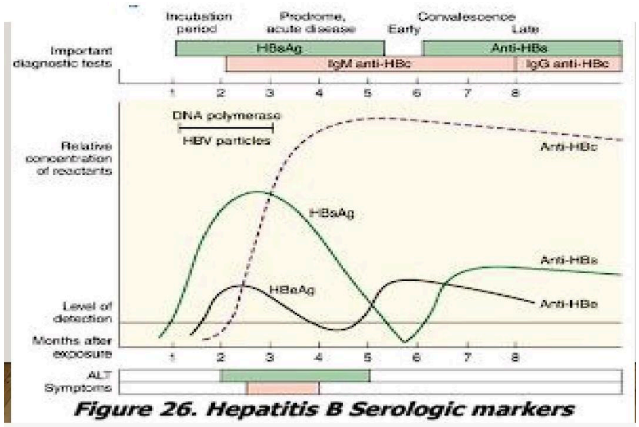


Figure 26. Hepatitis B Serologic markers

HBsAg (Hepatitis B Surface Antigen)

- most reliable marker for identifying HBV infection
- appears before the onset of symptoms
- It peaks during the symptomatic disease and declines usually to undetectable levels in about 12 weeks.
- Becomes evident even before the onset of liver damage such as the increase in liver function tests (AST, ALT, bilirubin)
- Acute (≤6 months) or chronic carrier (>6months)
- Decline of HBsAg – production of Anti-HBs patient is now in convalescent phase

- If Anti-HBs alone is detected – patient has been previously vaccinated or have developed immunity which persists forlife and confers protection against virus>
- HBeAg – appears after appearance of HBsAg, indicates ongoing viral replication, persistence means high infectivity and progression to chronic hepatitis

- Anti-HBe appearance – acute infection has peaked, is now weaning and approaching convalescent phase
- Anti-HBc (Antibody against core antigen) – can be seen even shortly before the onset of symptoms and in concurrence with elevated serum transaminase levels. Indicates hepatocyte damage/destruction
    - IgM – first antibody against core antigen, appears in the early course of the disease/acute.
    - IgG – replaces IgM in a few months to give immunity against the virus

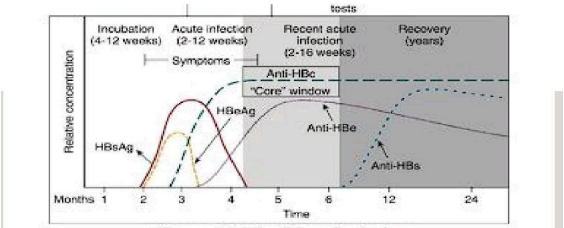


Figure 27. The "Core" window

Period in the infection when neither HBsAg and anti-HBs is detected in the serum of the patient. These two markers have reached their zone of equivalent circulation and are removed from the circulation. ***Only markers detected are Anti-HBc, Anti-HBe, and HBV DNA.***

TABLE 35-8 Interpretation of Hepatitis B Virus Serologic Markers in Patients with Hepatitis[a]

Assay Results			
HBsAg	Anti-HBs	Anti-HBc	Interpretation
Positive	Negative	Negative	Early acute HBV infection. Confirmation is required to exclude nonspecific reactivity
Positive	(±)	Positive	HBV infection, either acute or chronic. Differentiate with IgM anti-HBc. Determine level of replicative activity (infectivity) with HBeAg or HBV DNA
Negative	Positive	Positive	Indicates previous HBV infection and immunity to hepatitis B
Negative	Negative	Positive	Possibilities include: HBV infection in remote past; "low-level" HBV carrier; "window" between disappearance of HBsAg and appearance of anti-HBs; or false-positive or nonspecific reaction. Investigate with IgM anti-HBc. When present, anti-HBe helps validate the anti-HBc reactivity
Negative	Negative	Negative	Never infected with HBV. Possibilities include another infectious agent, toxic injury to liver, disorder of immunity, hereditary disease of the liver, or disease of the biliary tract
Negative	Positive	Negative	Vaccine-type response

[a]Modified and reproduced with permission from Hollinger FB: Hepatitis B virus. In Fields BN, Knipe DM, Howley PM (editors-in-chief), *Fields Virology*, 3rd ed. Lippincott-Raven, 1996.

Anti-HBc,antibody to hepatitis B core antigen; anti-HBe, antibody to hepatitis B e antigen; anti-HBs, antibody to hepatitis B surface antigen (HBsAg); HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; IgM, immunoglobulin M.

Figure 28. Interpretation of Hepatitis B Virus Serologic Markers in Patients with Hepatitis

Molecular Tests: PCR

- **HBV DNA** – best indication of active viral replication and high state of infectivity
    - Serum
- High analytical sensitivity
- Resolves any questionable serologic results
- Helpful for predicting patient's response to treatment