MYCOLOGY

Fungi are group of non motile eukaryotic (versus bacteria (prokaryotic)) organisms that have definite cell walls are devoid of chlorophyll, and reproductive by means of spores (and conidia) or budding.

Hetero = "different", trophic = "nourishment"

Mycosis (mycoses)
- Invasive treatment
- Immunosuppresive theraphy
- Immunocompromising infections (e.g. HIV)
- Rise in common and uncommon mycoses (organisms and tissue infected)

BASIC STRUCTURES

Cell wall – antigenic,multilayered (90% polysaccharide, chitin, 10% proteins and glycoproteins), provides shape and rigidity to the cell (facilitate osmosis).

Cell membrane - bilayered phospholipids, contains sterol (ergosterol and cholesterol), functions: protect the cytoplasm, regulates intake of nutrients and facilitates capsule and cell wall synthesis.

Mycelium – intertwining structure composed of tubular filaments known as hyphae.

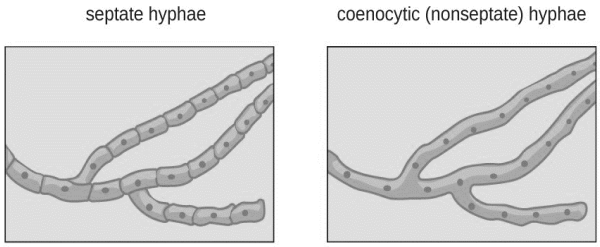
1. **Vegetative/ thallus** – grows in a substrate and absorbs nutrients.

2. **Reproductive/ aerial** – contains fruiting bodies that produce the conidia or spores, for reproduction.

Capsule – polysaccharide much larger than the bacterial capsule, virulence factor: antiphagocytic, mostly in yeast (e.g. *Cryptococcus neoformans*)

Cytoplasm – contains the nucleus, nucleolus, nuclear membrane, endoplasmic reticulum, mitochondria and vacuoles.

Hyphae – basic structural unit of fungi
1. **Septate hyphae** – with crosswalls/ divisions, present in all fungi EXCEPT in *Zygomycetes*
2. **Aseptate hyphae (Coenocytic)** - no crosswalls/ divisions, present in *Zygomycetes* (*Rhizopus, Absidia, Mucor*)



molds

Spores – for reproduction (sexual or asexual).

Spores involved in Sexual Reproduction

1.	**Ascospores**	Contained in a saclike ascus (2-8 ascospores)
2.	**Zygospores**	Involve the fusion of two identical cells arising from the same hyphae
3.	**Oospores**	Involve the fusion of cells from two separate, non identical hyphae
4.	**Basidiospores**	Contained in a club-shaped basidium

Spores involved in Asexual Reproduction

1. Conidia - Arise from side of hyphae - Catenate conidia; chains - Echinulate; rough, spiny	Spores produced singly or multiply in ong chains by specialized vegetative hyphae known as conidiophores
2. Blastoconidia (blastospores)	Develops as daughter cell buds off the mother cell and is pinched off

3. Chlamydoconidia (resting spores)	Thick walled, resistant, resting spores produced by rounding up and enlargement of terminal hyphal cells. The spores germinate when favorable environmental conditions occur 1. Terminal – end of hyphae 2. Intercalary - within hyphae 3. Sessile – side of hyphae
4. Arthroconidiaspores	Involve the simple fragmentation of the mycelium. Useful ID of *C. immitis* and *G. candidum*. Appears "jointed", rectangular/ barrel shaped spores
5. Sporangiospores	Spores contained in a sporangia or sacs that are produced terminally on sporangophore or aseptate hyphae. Unique to *Zygomycetes*.

FIVE CATEGORIES OF FUNGAL INFECTIONS

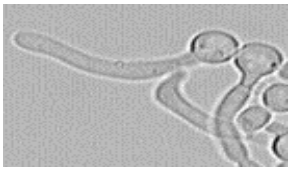
A. Opportunistic fungi

- Immunocompromised patients
- Many different tissues
- Ubiquitous: environmental saprobes
- Monomorphs
- Same structural characteristics under all conditions (e.g. *Candida, Cryptococcus, Aspergillus, Zygomycetes)*

1. *Candida albicans*

- Saprophytic in oral cavities, NF of the GI or vaginal tract, dimorphic
- Potential pathogen for immunosuppressed patients
- Infection: Candidiasis, Moniliasis (Oral – thrush; vaginal – vulvovaginitis)
  Onychomycosis (nails) Paronimycosis (cuticle)

Germ tubes are hyphae like extensions of young cells showing parallel sides, are non septate and will not constrict at their point of origin.
Pseudohyphae look like germ tubes but are septate and constricted at their point of origin.
Screening test: Germ tube test - serum + organism at 35°C for 2-3 hrs
(+) *C.albicans, C.dubliniensis* (-) *C.tropicalis*
Confirmatory test: Chlamydospore Corn meal
C.albicans to corn meal agar – Incubate at RT for 48-72 hrs. (+) Chlamydospores



2. *Cryptococcus neoformans*

- Encapsulated yeast cell in bird and bat droppings
- Demonstration of the capsule by India Ink stain
- Gram stain: "Starburst pattern"
- Infection: Torulosis
- Urease positive, inositol positive, nitrate positive
- Cultured on:
  SDA medium without cycloheximide
  Birdseed/ Nigerseed/ Staib's – brown black colonies due to phenol oxidase (assimilates creatinine) **Guizotia abyssinica*
- Latex agglutination test for antigen in CSF

3. *Aspergillus* - *Czapek's medium

- *Aspergillus fumigatus* – fungus ball
  Infection: Aspergilloma, allergy, otomycosis (bread mold) "Farmer's Lung disease"
- *Aspergillus flavus* –aflatoxin (toxicoses) *dried / processed nuts.
- *Aspergillus niger* – brown to black spore

4. *Pneumocystis jirovecii* (previously *P.carinii*)

- Mistaken as a protozoan because it has a trophozoite -> precyst -> cyst -> forms, no ergosterol

- **#1 cause of pneumonia in AIDS (PCP)**
- **#1 opportunistic infection in AIDS**
- Specimen for diagnosis: BAL (Broncho-Alveolar Lavage)

5. *Zygomycetes*

- Causative agents: *Rhizopus, Mucor* or *Absidia*
- Infection: Zygomycosis or Mucormycosis via inhalation of conidia

B. Superficial mycoses

- Infections of outer. "dead layers", person to person contact, fomites
- No host defense stimulation, pain or discomfort
- Usually treated because the infection is "unsightly"(e.g.*Malassezia, Piedraia, Trichosporon, Exophialia*)

1. *Malassezia furfur*

- Infection: Ptyriasis/ tinea versicolor – uneven pigmentation of the skin
- "spaghetti and meatballs" (PAS), (+) SDA with olive oil.

2. *Piedra Hortei*

- Infection: black Piedra_– brown-black crust outside hairshaft

3. *Trichosporon beigelii*

- Infection: white Piedra – light brown nodules on beard

4. *Exophiala werneckii*

- Infection: Tinea nigra - brownish spot, black macules

C. Dermatophytic/ Cutaneous mycoses

- Deeper than the superficial it affects keratinized tissue (skin, hair, nails), causes "tinea" latin word for ringworm.

Tinea capitis	Scalp
Tinea barbae	Beard area
Tinea corporis	Middle part (arms, trunk or legs)
Tinea manus	hands
Tinea cruris	"jock itch", groin area
Tinea unguium	Nails (yellow discoloration)
Tinea pedis	"athlete's foot"

- Still no living skin penetration
- Produce secondary metabolites that irritate host defense which causes itching
- Sometimes cutaneous and superficial grouped (e.g. *Trichophyton, Microsporum, Epidermophyton*)

1. ***Trichophyton* species**– skin, hair and nails

	Urease	Pigment	HPT
T. rubrum (tear drop microconidia)	(-)	red	(-)
T. mentagrophytes (grape-like micro-conidia)	(+)	(-)	(+) v shape
T.verrucosum	"rat tail or string bean shaped macroconidia "Clavate or pyriform" microconidia *Thiamine and Inositol		
T. tonsurans	"Balloon shaped microconidia" *thiamine		
T. shoenleinii	"Favic chandelier" hyphae		

*****HPT - Hair Perforation Test or Hair Baiting Test**

T. rubrum	Urease negative Macroconidia are pencil shaped Abundant wine-red water soluble pigment
T. mentagrophytes	Cigar shaped macroconidia Scant-red pigment in some strains
T. tonsurans	Macroconidia absent Microconidia with flattened base "balloon forms" – aged pleomorphic microconidia
T. verrucosum	Rat-tail macroconidia Microconidia are clavate
T. schoenleinii	Conidia absent Favic chandeliers and chlamydospores
T. violaceum	Conidia absent Swollen hyphae containing cytoplasmic granules

2. ***Microsporum* species**– skin and hair only

M. canis *(zoophilic)*	Large, multicelled, spindle shaped, rough macro-conidia Terminal ends

	(+) Wood's lamp - green/yellow fluorescence of ectothrix hairs (+) growth on rice grain medium
M. gypseum *(geophilic)*	3-9 celled, broadly spindle shaped (spindle), rough walled macro-conidia Oblong/ rounded terminal ends Microconidia in single/ small clusters
M. audouinii *(anthrophilic)*	Conidia absent Apple-green fluorescence of ectothrix hair (-) growth on rice grain medium

3. ***Epidermophyton* species** – skin and nails only

E. floccosum	Large multi-celled, club shaped, smooth walled macroconidia "Dutch pants fuseaux" Microconidia not formed

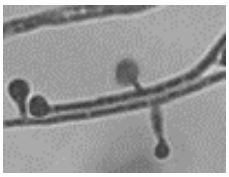
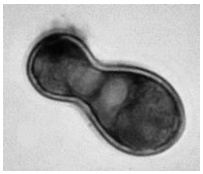
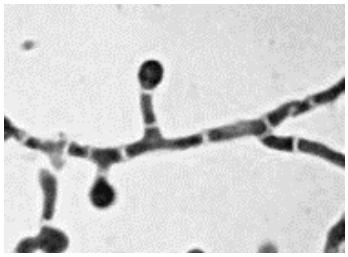
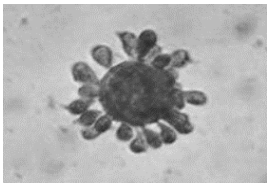
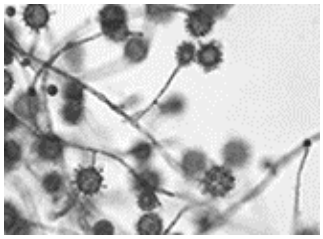
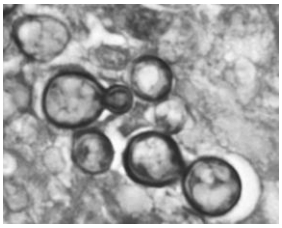
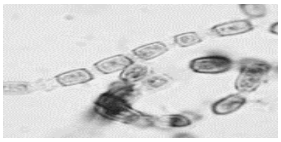
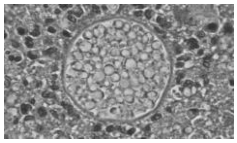
D. Subcutaneous mycoses

- Muscles, bone and connective tissues
- Trauma, inoculation, thorns or scratch
- Usually remain localized (*Chladosporium, Pseudallescheria, Phialophora, Exophiala, Sporothrix*)
- Isolated from soil
- Specimen: Biopsy stained with PAS or H&E (granules)

1. ***Fonsecaea pedrosoi***– mixed sporulation
   Phialophora verrucosa– vase like
   ***Cladosporum carrionii* –** cauliflower like lesion
   - Infection: Chromoblastomycosis
2. ***Pseudallescheria boydii, Madurella* and *Leptosphaeria* sp.**
   - Infection: Mycetoma or Madura foot– granulomatous tumor of subcutaneous tissue
   a. Eumycotic – true fungi; exophiala; most common cause is *Pseudoallescheria boydii*
   b. Actinomycotic – fungus like bacteria; do not stain with fungal stains; actinomycetes and nocardia
3. ***Rhinosporidium seeberi* (previously *Rhinocladiella aquaspersa*)**
   - Infection: Rhinosporidiosis – polypoid masses on the nose and the pharynx
   - Acquired through swimming
4. ***Loboa loboi***
   - Infection: Keloid like subcutaneous nodules involving the extremities.
5. ***Exophiala jeanselmei, Phialophora Wangiella dermatitidis***
   Cladosporum trichoides
   - Infection: Phaeohyphomycosis – rare infection caused by dematiaceous saprobes which invade organs of immunosuppressed hosts.
6. ***Sporothrix schenckii***
   - "rose gardener's disease"; dimorphic fungus
   - Infection: Sporotrichosis
   - Mold phase: flowerette conidia
   - Yeast phase: cigar shape *Asteroid body in tissue – central halo around degenerating yeast; concentric radiating eosinophilic material (Ag-Ab reaction).

E. Systemic mycoses

- "true"or "primary" pathogens that can infect any tissue
- Endemic to specific geographic areas. Must travel through area to become infected (inhalation)
- Thermal dimorphs and yeasts ("two bodies" based in temperature)(e.g. *Blastomyces, Paracoccidiodes,Histo-plasma, Coccidiodes*).

ORGANISM/ DSE	MOULD FORM (25°C)	YEAST FORM (37°C)
Blastomyces dermatitidis Infection: "North American Blastomycosis" "Chicago disease" "Gilchrist's disease" ***Cottonseed Agar**	Delicate, septate hyphae with round or pyriform conidia born singly on conidiophores resembling **lollipops** 	Thick walled, large yeast cells with single bud on a **broad base** 
Paracoccidiodes brasiliensis Infection: "South American Blasto-mycosis" "Lutz Splendore- Almeida disease"	Small, septate, branched hpahe with intercalary and terminal chlamydoconidia 	Large, round to oval, thick walled yeast cells with multiple buds which attach to mother cell by narrow constrictions, resembles a ship wheel, mariner's wheel or a Mickey Mouse cap 
Histoplasma capsulatum *Leishmania – mistaken for Histoplasma; differentiated by a central nuclear body and failure to stain with fungal stains Infection: "Darling's disease" "Spelunkers'disease" "Fungus Flu" ***isolated from droppings of bats – "guano" and birds (Starlings)	Septate hyphae with round to pyriform microconidia on short branches or directly on hyphal stalk , thick walled knobby tuberculated macroconidia forms 	Small, budding round to oval yeast cells, intracellular to mononuclear cells possible with Giemsa or Wright's stain 
Coccidioides immitis major biologic hazard to lab personnel Infection: "Coccidiomycosis" "San Joaquin Valley fever" "Desert fever"	Coarse, septate, branched hyphae that produce thick walled barrel-shaped rectangular arthroconidia that alternate with empty disjunctor cells 	Large, round, thick walled spherules with endospores observed in tissue and direct examination; not a true yeast 

LABORATORY SAFETY

- Standard precautions
- No smoking, eating, drinking or applying cosmetics
- Contact lenses (no removing or cleaning)
- No mouth pipetting
- Universal precautions
- Class 2 or 3 biosafety hoods
- Disinfectant: phenol based
- Biohazard containers

SPECIMEN COLLECTION

- Important factors in isolating and identifying a fungal pathogen
- Correct type of specimen
- Quality of specimen
- Rapid transport
- Use of appropriate culture media
- Processed within two hours

SPECIMEN TRANSPORT

- Sterile, leak-proof container
- Dermatologic requires dry container
- No transport media, processed within a few hours
- Specimen can be refrigerated at 4°C, only if processing is delayed
- Blood and CSF: 30 to 37°C
- Dermatologic: 15 to 30°C

SPECIMEN PROCESSING: METHODS

1. Direct inoculation
   - Adding several drops of specimen to media
   - For solid media, the specimen can be streaked
   - Specimen types: Bronchial brush or wash, aspirates, CSF, swabs, body fluids, hairs, scrappings
2. Concentration
   - Large volumes can be concentrated by centrifugation
   - Specimen types: body fluids, CSF, urine
3. Minced (homogenized)
   - Some old specimens must be "destroyed" to expose a buried pathogen to the media
   - Specimen typed: nails, tissues, biopsies
4. Culture of fungi
   - petri dishes or tubes (O_2 requirements, humidity)
   - subculture sometimes necessary (temperature range, dimorphism and sexual and asexual developmental structures)
   - Culture is very important can be main identification
   - No or few biochemical (yeast are the exception)
   - Get rid of bacterial contamination (teasing needles are used more than bacteriologic loops O - electric incinerator
     X - flame causes aerosols

CULTURE MEDIA

- Incubated at 30°C/ room temp, culture held for 21 days
- Test tube for primary - less likely to become contaminated, less drying.
- Petri dishes for subculture - Larger surface for area growth.
- Use of inhibitory substances may be required (e.g. Chloramphenicol, gentamicin, cyclohexamide), May encounter some fungal inhibition

Common Media for Isolation

1. Saborauds Dextrose Agar (SDA) - most commonly, most fungi grow well

2. Emmon's modification: less glucose (e.g. *Blastomy-ces dermatitidis*)

3. Mycosel and mycobiotic - SDA + Chloramphenicol + Cycloheximide, selective recovery of dimorphs and dermatophytes

4. Brain Heart Infusion (BHI) Agar - enriched to enhance recovery *Cryptococcus neoformans* of and dimorphic transitions in *Sporothrix* and *Paracocci-diodes*

- Plates or tubes
- Broth + penicillin for *Zygomycetes*
- BHI + gentamicin + chloramphenicol, *Cryptococcus neoformans* from contaminated specimen

4. Saboraud dextrose + BHI (SABHI)

- strengths of both
- enriched medium for *Cryptococcus* spp. Thermally dimorphic fungi, etc.

5. CHROMagar

- Selective and differential for presumptive identification of genus *Candida* from primary plates.

- Morphology and colors of the yeast colonies varies in species.
  - *Candida albicans* –light to medium green
  - *Candida tropicalis* – light blue to metallic-blue
  - *Candida krusei* – light rose with a whitish border

6. Inhibitory Mold Agar (IMA)
- Inorganic salts, chloramphenicol, gentamicin
- Inhibits bacteria

7. Dermatophyte test medium (DTM)
- Dermatophytes from heavily contaminated specimens (pink-to-red color change)
- Commonly used in office practices

8. Czapek's agar
- for *Aspergillus sp.*

9. Birdseed/ Nigerseed/ Staib's medium
- *C. neoformans* appear black colonies due to phenol oxidase

10. Cottonseed medium
- for *Blastomyces dermatitidis*

11. Rice medium
- differentiates *M. canis* (+) from *M. audouinii* (-)

Common Media for Subculture

1. Potato Dextrose Agar (PDA)/ Potato Flake Agar (PKA)

Incubation:
- Obligate filamentous: 25-37°C
- Dimorphic: 25°C and 37°C
- Yeast: 25°C or 37°C
- Aerobic, 3 to 4 weeks

2. Cornmeal Agar for yeast morphology – recommended for promoting sporulation
- *Candida* to visualize chlamydoconidia
- 1% dextrose: used to differentiate *T. rubrum* (red) from *T. mentagrophytes*

LABORATORY IDENTIFICATION

DIRECT EXAMINATION
- Can identify yeast and filamentous forms
- Culture is used regardless

1. KOH preparation
   - Examine hair, nails, skin scrappings, fluids, exudates, and biopsy specimen
   - Can see important fungal elements (hyphae, yeast)
   - Need reduced light or phase-contrast
   - 10% KOH added to specimen
   - Dissolves specimen quickly (fungi slowly)
2. Calcoflour white
   - Binds the cell wall and fluoresces blue-white under UV light
3. Lactophenol cotton blue (Aman's medium)
   - used to stain and preserve fungal elements in culture isolates
4. India ink
   - Used with CSF specimens
   - Negative stain
   - Creates black background to visualize capsular material (*C. neoformans)*
5. Tissue examination: stains
   - Gram stain (Hucker's modification) – for yeast (gram positive)
   - Giemsa, Wright-Giemsa: (intracellular) to locate H. capsulatum in RE cells
   - Hematoxylin and Eosin (H&E): Pink to pinkish-blue
   - Meyer's mucicarmine: *C.neoformans* – rose red
   - Gomori Methanamine Silver (GMS): black
   - Papanicolau stain: pink to blue
   - Periodic Acid Schiff (PAS): red or purple
   - Acridine orange: green fluorescence - fungal elements/ Orange for epithelial cells

MACROSCOPIC EXAMINATION

Growth conditions
- Yeast: 2-3 days
- Molds
  Rapid: Less than 5 days
  Intermediate: 6 to 10 days
  Slow: more than 11 (sometimes 8 weeks)

Dimorphism
- Pigment
- Front versus back of the plate
- Texture
- Dictated the presence of aerial hyphae
  - Glabrous: leathery or waxy
  - Velvety: Suede, plush
  - Yeast like: looks like *Staphylococcus*
  - Cottony: Fluffy
  - Granular: powdery

Topography
- Rugose: radial grooves, "folded"

- Crareriform: central depression and raised edge
- Verrucous: Rough knobs
- Cerebriform: Brainlike

Examination of Molds

Three methods

1. Tease/cut preparation: organism removed directly from culture plate, "teased" apart with teasing needles.

2. Scotch tape preparation: Scotch tape pressed onto culture plate then transferred to microscope slide.

3. Slide culture: organism is subcultured on a small piece of agar then covered with a cover slip. **Organism grows onto the coverslip: remove and examine. Best method.**

Examination of molds: Use one of the three methods listed previously, followed by the addition of stain, Lactophenol Cotton Blue (LPCB).

Dermatophyte Identification

1. Hair perforation test
   - 5 to 10-mm sterile hair floated on sterile water and yeast extract. Conidia or hyphae
   - inoculated onto water surface. Remove hair shafts and observe in LPCB weekly for 1 month

**Trichophyton mentagrophytes*: (+)
***Trichophyton rubrum*: (-)

2. Urease test
   - Tubes of urease agar are lightly inoculated for 5 days at room temperature

**Trichophyton mentagrophytes*: (+)
***Trichophyton rubrum*: (-) or weak

3. *Trichophyton* Agars
   - Originally numbers 1 to 4
   - Most laboratories use only 1 and 4

Thiamine requirement:
   - *Trichophyton* agar 1 (without thiamine)
   - *Trichophyton* agar 4 (with thiamine)
   - 10 to 14 days, observe for growth.

4. Rice grain growth
   - Sterile, nonfortified rice grain media, 10 days, observe for growth

**Microsporum canis*: (+)
***Microsporum audouinii*: (-)

5. L-DOPA ferric citrate test
   - for phenol oxidase of *C. neoformans* (+) black

Mold Identification

- Specimen source or infection
- Growth rate to reproduce structures
- Colony color front and back on plate
- Microscopic morphology
  - Septate or aseptate hyphae
  - Conidiophore structure
  - Microconidia/macroconidia
  - Other structures

Advanced Techniques

1. Exoantigen test
   - Rapid information of immnunoidentity
   - Extract soluble antigen from unknown isolate, concentrate
   - React with antiserum specific to known fungi
   - Positive control necessary for definitive identification
   - Test is read at 24 hrs

1. A antigen – *B. dermatitidis*
2. 1, 2 & 3 antigen – *P. brasiliensis*
3. H and M antigen – *H. capsulatum*
4. HS, HL and F antigen - *C. immitis*

2. DNA probe
   - Rapid kits that use nucleic acid hybridization to identify fungi in culture.
   - Highly specific to each fungus, because it is based on DNA sequence.
   - Needs to be performed on cultured organisms – not for specimens
   - Developed for *Coccidiodes, Blastomyces, Histoplasma*
   - Specialized clinical laboratories are using DNA sequ-encing techniques to establish fungal infections.

Yeast Identification

MACROSCOPIC MORPHOLOGY

- Colony color and texture
- Color: white, tan, pink, salmon
- Can have dematiaceous yeasts
- texture: mucoid, butterlike, velvety, wrinkled

MICROSCOPIC MORPHOLOGY: wet preparation

- Hyphae
- Pseudohyphae
- Blastoconidia

Cornmeal Tween 80 Agar

- Encourages development of chlamydospores
- Relationships among hyphae, pseudohyphae, and others
- Clear media: can be observed under light microscope
- Specific organisms associated with specific morphology
- Cornmeal agar morphology - used in conjunction with carbohydrate usage

Four main morphology types

1. Hyphae
2. Pseudohyphae
3. Arthroconidia
4. Chlamydioconidia or blastoconidia

a. Pseudohyphae or Blastoconidia only
Candida krusei
Candida parapsilosis
Candida keyfr
Candida tropicalis

b. Blastoconidia only
C.glabrata and *C.neoformans*

c. Arthroconidia - *Trichosporon beigelii*

PHYSIOLOGIC TESTS

1. Germ tube test

- Filamentous outgrowth of blastoconidia.
- Most basic and easiest to perform.
- Requires the use of serum or plasma.
- Some commercially made broths (will last longer). Over-incubation and over-inoculation are biggest problems.
- Other agents can form germ tubes
- Not valid if not read after 2 hrs.
- "true" germ tube: *C. albicans*
- No constrictions are base, where the tube attaches to the mother cell.
- constriction base indicates *C. tropicalis*
- Other species have germ tubes
  - *C. stellatoidea* (sucrose assimilation is used to differentiate from *C. albicans*)
  - *C. dubliniensis* (no growth at 4 hrs.)
- Positive and negative controls are necessary.

2. Fermentation/Assimilation

- Fermentation – carbohydrate is use in the absence of oxygen
- Assimilation – which can be used as a sole carbon source

Two Systems (Assimilation)
a. API 20C: strip test
b. Vitek: automated

3. Urea hydrolysis

- Detected on simple urea agar
- Rapid and easy
- Differentiate *Cryptococcus* from *Rhodococcus*
- Positive: pink, negative: little or no change

4. Temperature studies

- *Cryptococcus* sp.: weak growth at 35°C and no growth at 42°C
- *Candida sp.*: several can grow well exceeding 45°C

Order of events

1. Wet preparation
2. Germ tube: germ tube negative and from sterile site
3. Cornmeal Agar morphology
4. Physiologic/Biochemical reaction
5. Temperature

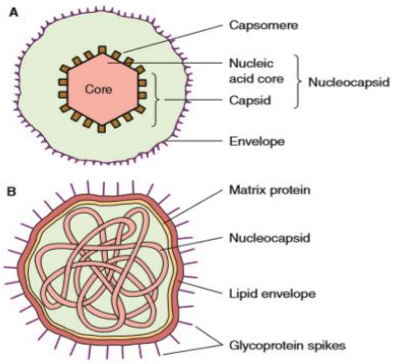
VIROLOGY

Viruses are the smallest infectious agents (ranging from about 20 nm to 300 nm in diameter), contain only one kind of nucleic acid (RNA or DNA).

Inert in the extracellular environment.

They replicate only in living cells (obligate intracellular parasites).

BASIC STRUCTURES



Capsid - the protein shell, or coat, which encloses the nucleic acid genome.

Capsomeres - morphologic units seen in the electron microscope on the surface of icosahedral virus particles. Capsomeres represent clusters of polypeptides, but the morphologic units do not necessarily correspond to the chemically defined structural units.

Envelope - a lipid-containing membrane that surrounds some virus particles. It is acquired during viral maturation by a budding process through a cellular membrane. Virus encoded glycoproteins are exposed on the surface of the envelope. These projections are called peplomers.

Virion - the complete virus particle.

a. Herpesviruses and Orthomyxoviruses: this includes the nucleocapsid plus a surrounding envelope. This structure, the virion, serves to transfer the viral nucleic acid from one cell to another.

b. Papillomaviruses and Picornaviruses: the virion is identical with the nucleocapsid.

PRINCIPLES OF VIRUS STRUCTURE

Electron microscopy

- Use of heavy metal stains (e.g., potassium phosphotungstate) to emphasize surface structure. The heavy metal permeates the virus particle like a cloud and brings out the surface structure of viruses by virtue of "negative staining."
- The typical level of resolution is 3–4 nm. (The size of a DNA double helix is 2 nm.) However, conventional methods of sample preparation often cause distortions and changes in particle morphology.

Cryoelectron microscopy

- Uses virus samples quick frozen in vitreous ice; fine structural features are preserved, and the use of negative stains is avoided.
- Three-dimensional structural information can be obtained by the use of computer image processing procedures.

X-ray crystallography

- Can provide atomic resolution information, generally at a level of 0.2–0.3 nm.
- The specimen must be crystalline, and this has only been achieved with small, nonenveloped viruses.

Genetic economy requires that a viral structure be made from many identical molecules of one or a few proteins. Viral architecture can be grouped into three types based on the arrangement of morphologic subunits:

1. Cubic symmetry (e.g., Adenoviruses) - icosahedral pattern, the most efficient arrangement for subunits in a closed shell. Viral structures built from repeated identical protein subunits to form a nearly spherical structure with rotational symmetry.

2. Helical symmetry (e.g., Orthomyxoviruses) - protein subunits are bound in a periodic way to the viral nucleic acid, winding it into a helix. Viral morphology composed of a single type of capsomer stacked around a central axis to form a helical structure.
3. Complex structures (e.g., poxviruses) - a virus that possesses a capsid that is neither truly helical nor icosahedral. This morphology may have additional structures such as a protein tail or a complex outer wall.

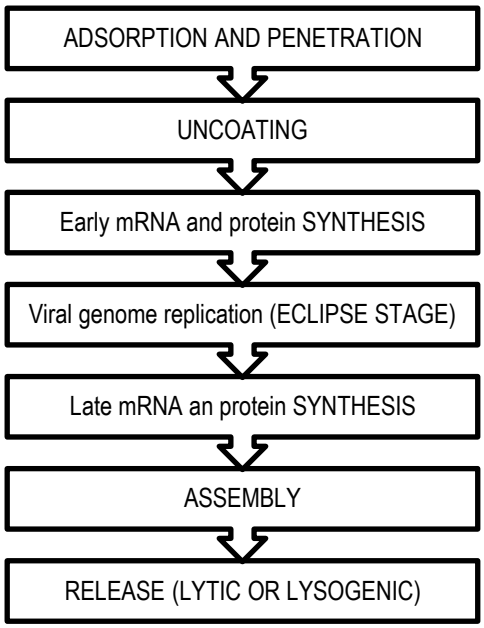
MEASURING THE SIZE OF VIRUSES

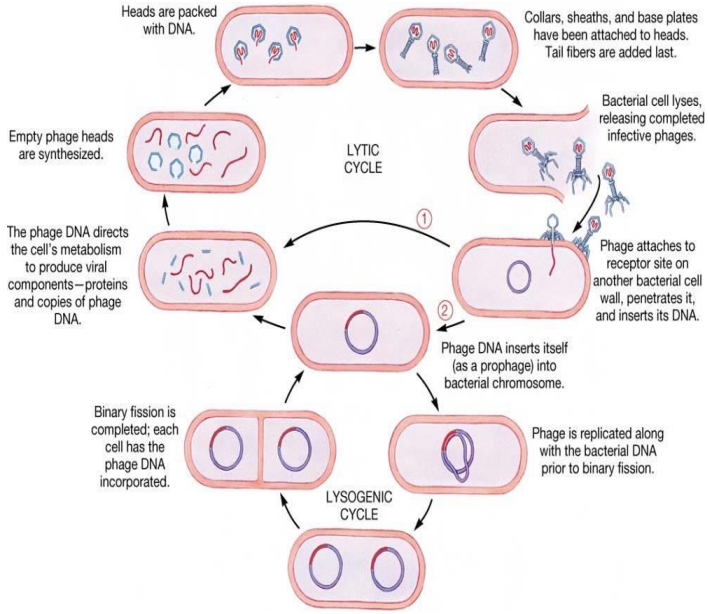
- Viruses are filterable pathogenic agents.
- Direct observation in the electron microscope is the most widely used method for estimating particle size. (preparations from tissue extracts and in ultrathin sections of infected cells).
- Another method that can be used is sedimentation in the ultracentrifuge.

CHEMICAL COMPOSITION OF VIRUSES

1. **Viral Protein:** surface proteins, enzymes
2. **Viral Nucleic Acid:** either DNA or RNA that encodes the genetic information necessary for replication of the virus.
3. **Viral Lipid Envelopes:** lipid envelopes as part of their structure.
4. **Viral Glycoproteins:** Viral envelopes contain glycoproteins e.g. influenza virus membrane glycoproteins (hemagglutinin, neuramini-dase).

MULTIPLICATION CYCLE





Proto-oncogene defines a normal gene that can become an oncogene due to mutations or increased expression. These genes commonly code for proteins involved in the regulation of cell growth or differentiation.

Sense is a concept used to compare the polarity of nucleic acids.

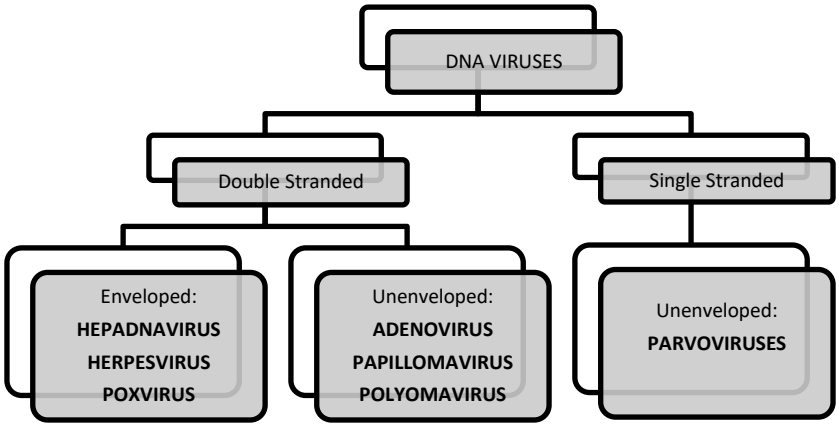
- Positive sense – 5' to 3', Positive-sense RNA (5' to 3') signifies that a particular sequence can be directly translated into protein.
- Negative sense – 3'to 5', Negative-sense RNA (3' to 5') forms the complementary strand and must be converted to positive-sense by an RNA polymerase prior to translation.

TYPES OF VIRUSES

DNA VIRUSES

Characteristics:

- All are D-S DNA except Parvovirus
- Replicate in the nucleus of the host cell except POXVIRUS (cytoplasm)
- Capsid: icosahedral or complex (nonconforming symmetry). All are ICOSAHEDRAL except POXVIRUS (complex)
- All are ENVELOPED (ether sensitive) except PAPOVAVIRUS, ADENOVIRUS AND PARVOVIRUS



FAMILY	CHARACTERISTICS	VIRUS	DISEASE	DIAGNOSIS/ COMMENTS
Adenoviridae	dsDNA genome; icosahedral capsid, unenveloped, 50 human serotypes	Adenovirus	*pharyngitis, *keratoconjunctivitis *pneumonia, *gastroenteritis in children	CPE in cell lines (swollen grapelike clusters)
Hepadnaviridae	Party dsDNA genome, icosahedral, enveloped Dane particle virion (David Dane)	Hepatitis B virus	*acute infection with resolution (90%), fulminant hepatitis most co infected with delta virus *chronic hepatitis persistence of HBsAg,	ELISA/PCR Antigens HbsAg – EXPO-SURE*carrier state

	Hepatitis B surface antigen termed as Australia antigen (Baruch Blumberg) Hepatitis D co-infection		followed by resolution, asymptomatic carries start *serum hepatitis	HBeAg – INFECTIVITY (HIGH) HBcAg Anti-bodies *Anti-HBcAg – IgM (Acute) IgG (Chronic) *window period *Anti-HBeAg *Anti-HBsAg - Vaccination
Herpesviridae		Herpes Simplex Virus – 1 *latency site: Trigeminal ganglion	*primary infection - Gingivostomatitis, *Latency infection - pharyngitis, herpes labialis, keratitis, encephalitis	Tzanck smear – Giemsa stained, scraping from the base of the vesicle Immunofluore-scent = (+) multinucleated giant cells with cowdry Cell culture – most diagnostic, CPE occur in 1-5 days – Identification by IFT
		Herpes Simplex Virus – 2 *latency site: Sacral ganglion	*genital herpes *neonatal herpes *aseptic Meningitis *cervical CA	
		Herpes Simplex Virus – 3: Varicella – Zoster Virus *latency site: Dorsal root of ganglia	*primary infection: Chicken pox (Varicella) *Latency: Shingles (Zoster) *Reyes syndrome – induced by aspirin	Tzanck smear - multinucleated giant cell w/ cowdry type inclusions (HSV, HZV) IFT- method of choice Lesions cultured on A-549

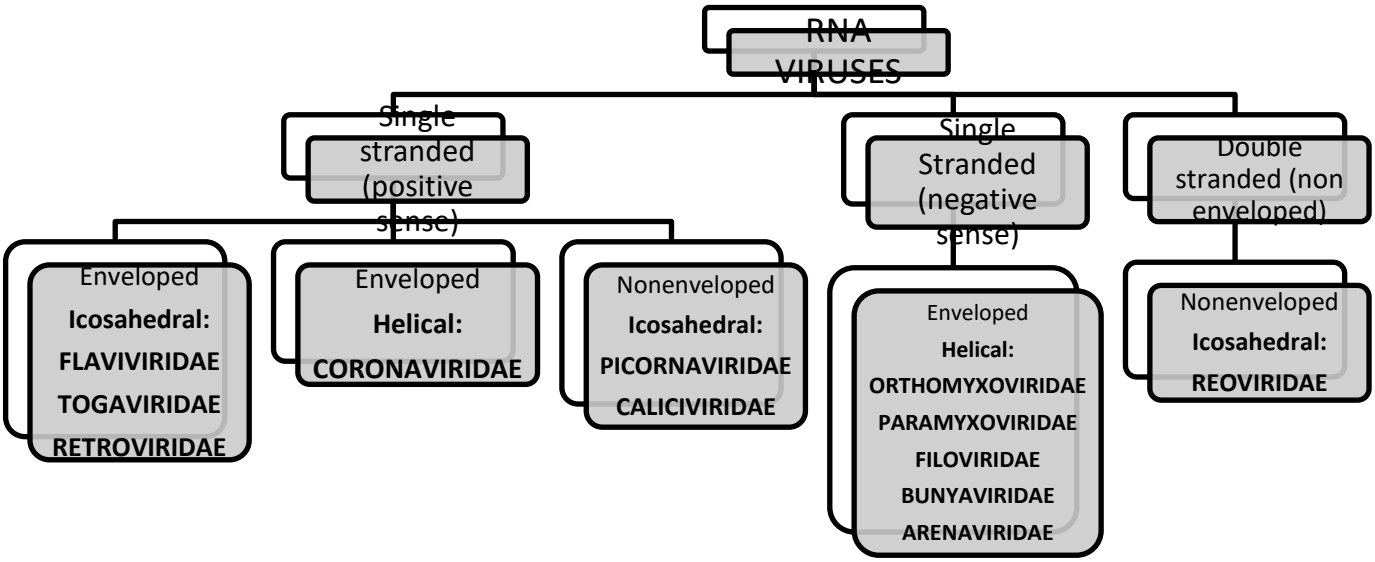
		Herpes Simplex Virus - 4 Epstein-Barr virus *infects B cells *Heterophile Abs	*Infectious mononucleosis *Progressive lymphoreticular disease *Oral hairy leuko-plakia in HIV patients *Burkitt's lymphoma *Glandular fever or Nasopharyngela CA	Atypical lymphocytes, Paul-Bunnell Test) Heterophile antigen test: Monospot test EBV specific Ab test: EBVCA IgM, EBNA Hematology: Downey cells
		Herpes Simplex Virus – 5 - Cytomegalovirus "Salivary gland virus"	*Congenital disease of the newborn, 40-day fever *mononucleosis like syndrome	Blood (buffy coat) Body fluids, urine, tissues, respiratory secretions cultured on HDF (Owl's eye inclusion) – Giemsa/PAP
		HSV-6 HSV-7 - X	*Roseola (exanthem subitum), 6th childhood disease, malaise, rash, fever, leukopenia	T -cells
		HSV-8	*Kaposi's sarcoma (AIDS- px) - purple rashes on skin, cause of cervical CA	Non-culturable
Papillomaviridae		Human Papilloma Virus	*Tropism for squa-mous epithelium – "warts (verrucae)" – HPV 6 and HPV 11 Disease: "Condylomata acuminata - ano-genital" Cervical Squamous, Vulvar or Penile CA – HPV 16 and HPV 18	Nonculturable Pap smear for koilocytes HPV vaccine
Polymaviridaea (Human Papovavirus)		BK virus (B.K. initials - Px)	Virus remains dormant in kidneys, reactivation in immunecompro-	

		JC virus (John Cunningham - px)	mised patients causes hemorrhagic cystitis *Progressive multifocal leukoencephalopathy	
Parvovirus	ssDNA, icosahedral, no envelope, parvovirus B-19 is the only human parvovirus	Parvovirus B-19	*Erythema infectiosum (5th disease) Slapped cheek rash *Hydrops fetalis – miscarriages,	Smallest DNA virus PCR/viral DNA from blood or amniotic fluid
Poxvirus	Largest and most complex of all viruses, brick shaped with nonconforming symmetry referred to as complex, dsDNA	Smallpox virus	Smallpox is a generalized infection with pustular rash; molluscum manifests as benign modules of the skin. *Smallpox (variola major) *Alastrim (variola minor) *Molluscum, contagiosum – wart like tumors, vaccinia viruses *Monkeypox and Orf	CPE on culture or Pocks on chorioallantoic membrane
Iridoviridae	dsDNA, icosahedral	Iridovirus	*African Swine fever virus	
Anelloviruses	small (~30 nm in diameter), icosahedral virions that lack an envelope. The viral genome is circular, single-stranded DNA, 2.0–3.9 kb in size.	Torque Teno virus	* No specific disease associations have been proven. There is limited knowledge about viral gene expression and replication.	

RNA VIRUSES

Characteristics:

- All are S-S RNA except REOVIRUS. Replicates in the cytoplasm of the host cell.
- Generally HELICAL or ICOSAHEDRAL except the POSITIVE SENSE RNA VIRUS
- All are ENVELOPED except PICORNAVIRUS (smallest), CALICIVIRUS AND REOVIRUS
- All are NON-SEGMENTED except REOVIRUS, ORTHOMYXOVIRUS, BUNYAVIRUS and ARENAVIRUS



FAMILY	CHARACTERISTIC	VIRUS	DISEASE
Arenaviridae	Enveloped, irregular capid, with 2 segmented ssRNA genome Arena – "sandy" appearance	Lymphocytic choriomenin-gitis virus (LCM) Lassa Fever virus (zootic -rats) Machupo virus Junin virus Sabia virus	*LCM causes asymptomatic to influenza-like to aseptic meningitis-type disease, *Bolivian Hemorrhagic fever *Argentinian Hemorrahagic fever *causes influenza like disease to severe hemorrhagic fever
Astroviridae	Characteristic six point star like structure Isolated among chicken, ducks and turkeys	Astrovirus	*Gastroenteritis among young children/ diarrhea outbreaks
Bunyaviridae	Segmented, ssRNA genome, spherical or pleomorphic capsid with envelope	**Arbovirus:** California Encephalitis Virus La Crosse Virus	*Encephalitis for arbo-viruses, pneumonia or hemorrhagic fever for hantaviruses

		Non-arbovirus: Sin Nombre Virus – "Four Corners Virus" - rodents Hantaan River Virus/ Korean Hemorrhagic Fever – field mice (Korean War) Rift Valley Fever virus - raw milk (cattles, goats and buffaloes) – Rift Valley, Kenya Crimean Congo hemor-rhagic Fever virus	
Caliciviridae	Nonenveloped, icosahedral capsid surrounding ssRNA genome	Norwalk – "Winter Vomiting bug": Norwalk, Ohio Hepatitis E virus	Nausea, vomiting and diarrhea, outbreaks of gastroenteritis in schools, colleges, nursing homes, cruise ships (Norwalk) *Hepatitis (water borne hepatitis) similar to that caused by heap A except for extraordinarily high case fatality rate among pregnant women
Coronaviridae	ssRNA, helical capsid with envelope large halo or crown like structure	Coronavirus Severe Acute Respiratory Syndrome Corona Virus Middle Eastern Respiratory Syndrome Corona Virus Novel Corona Virus- 2019	*Common cold, possibly gastroenteritis, especially in children Novel Human Corona Viruses 1. HCoV – 229E and HCoV – OC43 2. NL63 – New Haven CoV (2004) 3. Human Corona Virus HKU1 (2005) 4. MERS – CoV (2012 and 2013) 5. MERS – CoV (2015 - Korea) 6. nCoV-19 (2019 -Wuhan)
Filoviridae	enveloped, long, fila-mentous, and irregular capsid forms with ssRNA	Ebola-Marburg viruses – Shepherd's crook, "U" or "6" shaped virus (fruit bats/bush meat/other pri-mates)	*Severe hemorrhage and liver necrosis

Flaviviridae	ssRNA, genome, surroundded by spherical and icosahedral capsid with envelope	**Arboviruses** Dengue virus (*Aedes aegypti*) West Nile Encephalitis virus (bird-mosquito-man) Japanese B Encephalitis virus (pig-mosquito-man) St. Louis Encephalitis viruses (*Culex*) Zika virus - mosquito	*acute hemorrhagic fever, "saddleback fever or breakbone fever" *Yellow fever *microencephaly
		Hepatitis C virus/ NANB virus Hepatitis G virus	*Post Transfusion Hepatitis, strong correlation between chronic HCV infection and hepatocellular carcinoma
Orthmyxoviridae	Segmented, ssRNA, helical, with envelope with spikes Hemeagglutinin (H) – 16 Neuraminidase (N) - 9 Antigenic changes 1. Shift (genetic reassortment) - Pandemic 2. Drift (point mutation) - epidemic	Influenza A Influenza B Influenza C	*Pandemic (Antigen shift and drift) – humans, birds and pigs AH1:N1 – Spanish flu (1918) and Swine flu (2009) AH2:N2 – Asian flu AH3:N2 – Hongkong flu AH5:N1 – Avian flu, pandemic threat *Epidemic (Antigen drift) – humans and seals *humans, pigs and dogs Flu Vaccine
Paramyxoviridae	Single-stranded, helical, non-segmented, enveloped Hemeagglutinin (H) Neuraminidase (N) Fusion (F)	Measles virus (Rubeola/ Morbilivirus)	Koplik's spots in buccal mucosa Atypical measles occurs in those with vaccine immunity Subacute sclerosing panencephalitis
		Mumps	*Mumps/Parotitis (rare cause of orchitis) – infertility in men

		Parainfluenza virus (common cause of pneumonia)	Adults: upper respiratory, rarely pneumonia Children: *Croup, bronchio-litis, pneumonia
		Respiratory syncytial virus (RSV) - common cause of pneumonia Henipavirus Nipah virus (bats) Hendra virus Metapneumovirus	Infants: bronchiolitis,pneumonia, croup Children: upper respiratory *Encephalitis (pig to man) *Respiratory disease horses *2nd after RSV that causes lower respiratory tract infection among children
Picornaviridae		Enteroviruses (acid resistant) Poliovirus Coxsackievirus A Coxsackievirus B Echovirus (Enteric Cyto-pathic Human Orphan virus) Enterovirus 68-71	 *Poliomyelitis (Vaccines: Inactivated: Jonas Salk; Live attenuated/oral: Albert Sabin) *Herpangina: Hand-foot-mouth disease *Pleurodynia: pericarditis, myocarditis (Devil's Grip disease) *Aseptic meningitis *Enterovirus 70: acute hemorrhagic conjunctivitis *Enteroviral meningitis
		Hepatitis A virus (enterovirus type 72)	*infectious hepatitis *Hepatitis with short incubation time, abrupt onset, low morality, no carrier state
		Rhinovirus – 33°C (acid sensitive)	*common cold/ Acute Viral Nasopharyngitis
Reoviridae	Segmented, dsRNA genome, icosahedral capsid, no envelope	Rotavirus – wagon-wheel like) Orbivirus/ Colorado Tick Fever Virus	Gastroenteritis in infants and children 6 months to 2 years (winter virus) Adults: asymptomatic

Retroviridae	ssRNA genome, icosahedral capsid with envelope, reverse transcriptase converts genomic RNA into DNA **Major Proteins** **gag** capsid protein (CA) – (p24) matrix protein (MA) – (p17) nucleoprotein (NC) – (p7) **pol** – (enzymes) reverse transcriptase (p51) integrase (p32) protease (p10) **env** gp160 (gp140 and gp41)	HIV-1 HIV-2 (African strain) Genus: lentivirus Human T lymphotropic viruses (HTLV-1 and 2) AIDS related Complex LymphoAdenopathy Associated Virus	Most diseases in humans is caused by HIV 1; infected cells include CD4, monocytes, asymptomatic infection, acute flu-like diseases, AIDS related complex, AIDS, AIDS associated malignancies T-cell leukemia and lymphoma, and tropical spastic paraparesis for HTLV-1, no known disease associated with HTLV-2 (isolated on patients with hairy cell leukemia) *screened in US bloodbanks due to high incidence in drug users
Rhabdoviridae	ssRNA genome, helical capsid with envelope, bullet-shaped with spikes	Rabies Lyssavirus (CNS infection in humans and animals)	*Rabies - latin "madness" * FAT of rabies antigen (dog brain), Seller's stain of Negri bodies
Togaviridae *reservoir: birds	icosahedral	Western, Eastern, and Venezuela Equine Encephalitis, Triple E or sleeping sickness Rubella virus	*zoonotic (equine - horses) *encephalitis * German measles (3-day rash), "blueberry muffin baby", Rubella – "little red" Teratogenic virus (1st week of pregnancy)

LABORATORY SAFETY

1. Aerosols: generated by homogenization of infected tissues, centrifugation, ultrasonic vibration, or broken glassware.
2. Ingestion: from mouth pipetting, eating or smoking in the laboratory, or inadequate washing of hands
3. Skin penetration: from needle sticks, broken glassware, hand contamination by leaking containers, handling of infected tissues, or animal bites.
4. Splashes: into the eyes or skin

Good biosafety practices include the following:

1. Training in and use of aseptic techniques
2. **X** of mouth pipetting
3. No eating, drinking, or smoking in the laboratory
4. Use of PPE (e.g., coats, gloves, masks) not to be worn outside the laboratory
5. Sterilization and decontamination.
6. Use of biosafety hoods (*Biosafety Level 4 when working with high-risk agents such as the filoviruses and rabies virus)
7. Immunization if vaccine is available

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

- Early stage of infection before the height of fever
- Sample the infected site
- Tissues and swabs require a viral transport medium; aspirates do not require transport medium
- DO NOT use CALCIUM ALGINATE swabs (only dacron, rayon, or cotton swabs)
- **TRANSPORT:** 4°C (1-2 days) - swabs
  - Stuart's Medium
  - Leibovitz-Emory
  - Earl/Hanks Balanced Salt Solution – Amino Acids, vitamins and bicarbonate, Penicillin-Streptomycin, Phenol Red
- **STORAGE:** -70°C (>3 days)

	Disease/ manifestation	Specimen	Common Agents
Respiratory tract	Croup, bronchiolitis, pneumonia	Nasal aspirate, NP swab, throat swab, BAL	Influenza, PIV, RSV, HSV, Adenovirus, Picornaviridae, Rotavirus
Gastrointestinal	Gastroenteritis	stool, rectal swabs	Adenovirus 40& 41, Enterovirus, Norwalk, Rotavirus
Cutaneous	Lesions and exanthems (rashes)	vesicle aspirate, lesion swabs	HSV 1,2,3, Adenovirus, Measles, Rubella, Enterovirus, Rotavirus
Ocular	Conjunctivitis, keratitis	conjunctival scrapings, corneal swabs/ scrapings	HSV, Adenovirus, Enterovirus
CNS	Encephalitis, aseptic meningitis	CSF, nasopahryngeal swabs	HSV, Arbovirus, Enterovirus, Mumps
Genital	Urethritis, penile lesions	vesicle aspirate or swab	HSV
Congenital	Lesions and exanthems	throat swab, urine, serum	CMV, HSV, Rubella
Disseminated	multiple	multiple sites	Adenovirus, Enterovirus, Rotavirus

CULTIVATION AND ASSAY OF VIRUSES

Cultivation of Viruses

Viruses can be grown in cell cultures or in fertile eggs under strictly controlled conditions (e.g. embryonated chicken eggs – Influenza, Tissue culture medium: AGMK (vero cells)*African green monkey cells, A-549 *Adeno-carcinoma human alveolar basal epithelial cell, WI-38*Winstar Institute – aborted fetus, MRC-5*Medical Research Council cell strain 5 – aborted Caucasian fetus – lung carcinoma cells).

Animals - primary isolation of certain viruses and for studies (pathogenesis of viral diseases and of viral oncogenesis).

- Serial passage – refers to the process of growing the bacteria or virus in iterations (repetitions).

There are three basic types of cell cultures

1. **Primary cultures** - dispersed cells (usually with trypsin) from freshly removed host tissues. In general, they are unable to grow for more than a few passages in culture (e.g. Primary Monkey Kidney Cells).
2. **Diploid cell lines/Secondary cultures** - have undergone a change that allows their limited culture (up to 50 passages) but that retain their normal chromosome pattern (e.g. Human Diploid Fibroblast).
3. **Continuous cell lines** are cultures capable of more prolonged/indefinite growth that have been derived from diploid cell lines or from malignant tissues. They invariably have altered and irregular numbers of chromosomes. The type of cell culture used for viral cultivation depends on the sensitivity of the cells to a particular virus (Madin Darby Canine Kidney, Hep-2 (Human Epithelial Cells type 2 – Laryngeal CA), HeLa – Henrietta Lacks – Cervical CA).

A. Detection of Virus-Infected Cells Multiplication of a virus can be monitored in a variety of ways:

1. Development of cytopathic - refers to the degenerative changes in association with viral infection.
    a. **Rounding necrosis** – Enterovirus
    b. **Ballooning/ Giant cell** – HSV
    c. **Grape like cluster** – Adenovirus
    d. **Syncytium form** – RSV/ Measles/ Rubella
    e. **Hemeadsorption** – Influenza, Parainfluenza, Measles and Mumps
    f. **Refractile, round cell** – Rhinovirus (33°C)
    g. **No CPE** – Influenza, Parainfluenza and Mumps

2. Appearance of a virus-encoded protein (e.g. hemagglutinin - influenza virus). Specific antisera can be used to detect the synthesis of viral proteins in infected cells. Hemagglutination - refers to the agglutination, or clumping, of red blood cells.

3. Detection of virus-specific nucleic acid (PCR).

4. Adsorption of erythrocytes to infected cells, called hemadsorption (e.g. hemagglutinin - parainfluenza, influenza) in cellular membranes

5. Viral growth in an embryonated chick egg may result in:
    - Death of the embryo (e.g., encephalitis viruses)
    - Production of pocks or plaques on the chorioallantoic membrane (e.g., herpes, smallpox, vaccinia)
    - Development of hemagglutinins in the embryonic fluids or tissues (e.g., influenza)

INCLUSION BODY FORMATION

- Observed through a light microscope (inverted microscope).
- They become far larger than the individual virus particle and often have an affinity for acid dyes (e.g., eosin).
- The presence of inclusion bodies may be of considerable diagnostic aid.

VIRUS	INTRACYTOPLASMIC EOSINOPHILIC	INTRANUCLEAR BASOPHILIC	INTRANUCLEAR EOSINOPHILIC
Rabies (fix with acetone)	Negri bodies		
Smallpox (Vaccinia, Variola)	Guarnieri bodies		
Smallpox (Variola)	Paschen bodies		
Fowlpox	Bollinger bodies		
Molluscum contagiosum	Henderson-Patterson bodies		
HSV			Cowdry Type A
VZV			Cowdry Type A
Yellow fever			Torres bodies
Polio and Adenovirus			Cowdry Type B
Adenovirus		Cowdry Type B	
CMV		Owl's Eye appearance	
Rubeola		Warthin-Finkeldey bodies	

QUANTITATION OF VIRUSES

A. Physical Methods

- **Quantitative nucleic acid-based assays** - Polymerase Chain Reaction (number of viral genome copies in a sample.)
- **Serologic tests** such as RadioImmuno-Assays (RIA), Enzyme-Linked Immuno-Sorbent Assays (ELISA) and Hemagglu-tination.

B. Biologic Methods

- **End point biologic assays** (e.g. measure-ment of animal death, animal infection, or CPE). Titer - 50% infectious dose (ID_{50}),reciprocal of the dilution of virus that produces the effect in 50% of the cells or animals inoculated.
- **Plaque assay** - tissue culture. Monolayers of host -> overlaid with medium containing agar or carboxymethylcellulose to prevent virus spreading throughout the culture. -> After several days, the cells initially infected have produced virus that spreads only to surrounding cells. Multiple cycles of replication and cell killing produce a **small area of infection, or plaque.**
- **Herpes and Vaccinia** - form pocks when inoculated onto the chorioallantoic mem-brane.

PURIFICATION AND IDENTIFICATION OF VIRUSES

Purification

1. Concentration by precipitation (e.g. ammonium sulfate, ethanol, or polyethylene glycol or by ultrafiltration) or hemagglutination and elution (Orthomyxoviruses).

2. Centrifugation
   a. Rate-zonal centrifugation: a sample of concentrated virus is layered onto a preformed linear density gradient (sucrose or glycerol) band at a rate by the size and weight of the virus particle.
   b. High-speed centrifugation in density gradients (e.g. cesium chloride, potassium tartrate, potassium citrate, or sucrose).

3. Column chromatography: virus is bound to a substance such as diethylaminoethyl or phosphocellulose and then eluted by changes in pH or salt concentration.

4. Zone electrophoresis permits separation of virus particles from contaminants on the basis of charge.

Identification of a Particle as a Virus

1. The particle can be obtained only from infected cells or tissues.

2. Particles obtained from various sources are identical regardless of the cellular origin in which the virus is grown.
3. Particles contain nucleic acid (DNA or RNA), the sequence of which is not the same as the species of host cells from which the particles were obtained.
4. The degree of infective activity of the preparation varies directly with the number of particles present.
5. Destruction of the physical particle by chemical or physical means is associated with a loss of viral activity.
6. Certain properties of the particles and infectivity must be shown to be identical (e.g., their sedimentation behavior in the ultracentrifuge and their pH stability curves).
7. Antisera prepared against the infectious virus should react with the characteristic particle and vice versa. Direct observation of an unknown virus can be accomplished by electron microscopic examination of aggregate formation in a mixture of antisera and crude viral suspension.
8. The particles should be able to induce the characteristic disease in vivo (if such experiments are feasible).
9. Passage of the particles in tissue culture should result in the production of progeny with biologic and antigenic properties of the virus.

REACTION TO PHYSICAL AND CHEMICAL AGENTS

Heat and Cold

- Icosahedral viruses: stable, losing little infectivity after several hours at 37°C.
- Enveloped viruses are much more heat labile, rapidly dropping in titer at 37°C.
- Enveloped viruses tend to lose infectivity after prolonged storage even at -90°C and are particularly sensitive to repeated freezing and thawing.
- Viral infectivity: **X** by heating at 50–60°C for 30 minutes except (e.g., Hepatitis B virus, polyomaviruses).
- Viruses can be preserved by storage at subfreezing temperatures, and some may withstand lyophilization (4°C or even at room temperature)
- Viruses that withstand lyophilization are more heat resistant when heated in the dry state.

Stabilization of Viruses by Salts

- Many viruses can be stabilized by salts in concentrations of 1 mol/L (i.e., the viruses are not inactivated even by heating at 50°C for 1 hour).
    - $MgCl_2$, 1 mol/L, stabilizes Picornaviruses and Reoviruses.
    - $MgSO_4$, 1 mol/L, stabilizes Orthomyxoviruses and Paramyxoviruses.
    - Na_2SO_4, 1 mol/L, stabilizes Herpesviruses.

***The stability of viruses is important in the preparation of vaccines.**

pH

- Viruses are usually stable between pH values of 5.0 and 9.0.
- Enteroviruses - resistant to acidic conditions.
- All viruses are destroyed by alkaline conditions.
- Hemagglutination reactions, variations of less than 1.0 pH unit may influence the result.

Radiation

- Ultraviolet, x-ray, and high-energy particles inactivate viruses.

Ether Susceptibility

- Enveloped Virus = sensitive to ether (ESE)
- Naked Virus = resistant to ether (NRE)
  ***lipids in the viral envelope is soluble to ether (fat solvent)**

Detergents

- Nonionic detergents (e.g., Nonidet P40 and Triton X-100) solubilize lipid constituents of viral membranes.
- Anionic detergents (e.g., sodium dodecyl sulfate) also solubilize viral envelopes; in addition, they disrupt capsids into separated polypeptides.

Formaldehyde

- Formaldehyde destroys viral infectivity by reacting with nucleic acid.

Photodynamic Inactivation

- Vital dyes (e.g. toluidine blue, neutral red, and proflavine). These dyes bind to the viral nucleic acid, and the virus then becomes susceptible to inactivation by visible light.
- Neutral red: commonly used to stain plaque assays so that plaques are more readily seen.

Antibiotics and Other Antibacterial Agents

- Antibacterial antibiotics and sulfonamides: **X** effect on viruses.
- Quaternary ammonium compounds (QUATS): in general, are not effective against viruses.
- Organic iodine compounds are also ineffective. Chlorine treatment -> stools adequate to inactivate typhoid bacilli is inadequate to destroy poliomyelitis virus present in feces.
- Alcohols: isopropanol and ethanol, are relatively ineffective against certain viruses, especially picornaviruses.

Common Methods of Inactivating Viruses for Various Purposes

- Sterilization: steam under pressure, dry heat, ethylene oxide, and γ-irradiation.
- Surface disinfectants: sodium hypochlorite, glutaraldehyde, formaldehyde, and peracetic acid.
- Antiseptics: skin disinfectants include chlorhexidine, 70% ethanol, and iodophores.
- Vaccine: use of formaldehyde, β-propiolactone, psoralen + ultraviolet irradiation, or detergents (subunit vaccines) to inactivate the vaccine virus.

PRIONS

- Proteinacious infectious virions or Transmissible spongiform encephalopathies (TSEs)
- Neurodegenerative diseases that affect both human and animals
- Target site: Brain – rapidly progressive and always fatal (Prions characteristics in the cells are holes – creating a sponge like tissue lesion)

Human Prion Disease

- Creutzfeldt-Jakob disease (CJD)
- Gertsmann-Straussler-Scheinker Syndrome
- Fatal familial insomnia
- Kuru

Animal Prion Disease

- Bovine Spongiform Encephalopathy (BSE)
- Chronic Wasting Disease (CWD)
- Scrapie
- Transmissible Mink Encephalopathy
- Feline Spongiform Encephalopathy
- Ungulate Spongiform Encephalopathy

REFERENCES:

Bailey & Scott's Diagnostic Microbiology, Elsevier 13th edition, 2014.
Jawetz, Melnick, & Adelberg's Medical Microbiology, McGraw-Hill 27th edition, 2014.
Graeter, Hertenstein, Accurso and Labiner, Medical Laboratory Science Examination Review, Elsevier, 2015.
"ATCC® MYCOLOGY CULTURE guide tips and techniques for culturing yeasts and filamentous fungi".
https://www.atcc.org retrieved April 22, 2018.
"ATCC® VIROLOGY GUIDE Tips and techniques for propagating virus in tissue culture and embryonated chicken eggs". https://www.atcc.org retrieved April 22, 2018.

/prdvrmt2020