SAWA UNIVERSITY

VIROLOGY

LABORATORY SAFETY &VIRAL IDENTIFICATION

جامعـــــــة ســـــــــاوة الاهلية — Sawa University

كليـــــة التقنيـــات الصحيـة والطبيـة — College of health and medical techniques

قسم تقنيات المختبرات الطبية — Department of Medical Laboratories

المرحلـــــــــــــة الثالثة — Three :Stage .

الجانب العملي ...محاضرة رقم 1

Practical Lecture No.1 ..

تدريسي المادة : م. د. سيف علي

safety in Virology Laboratory

Biosafety is: The application of combinations of laboratory practice and procedures, laboratory facilities, and safety equipment when working with potentially infectious microorganisms.

Biosafety designed to protect human health and prevent release of pathogens into the environment.

What are Biohazards or Biological hazards?

are organic substances that pose a threat to the health of humans and other living organisms, which includes:

Bio-active substances, viruses, spores or, fungi

Toxins and allergens derived from those organisms

Allergens and toxins derived from higher plants and animals

Other examples of biohazards:-

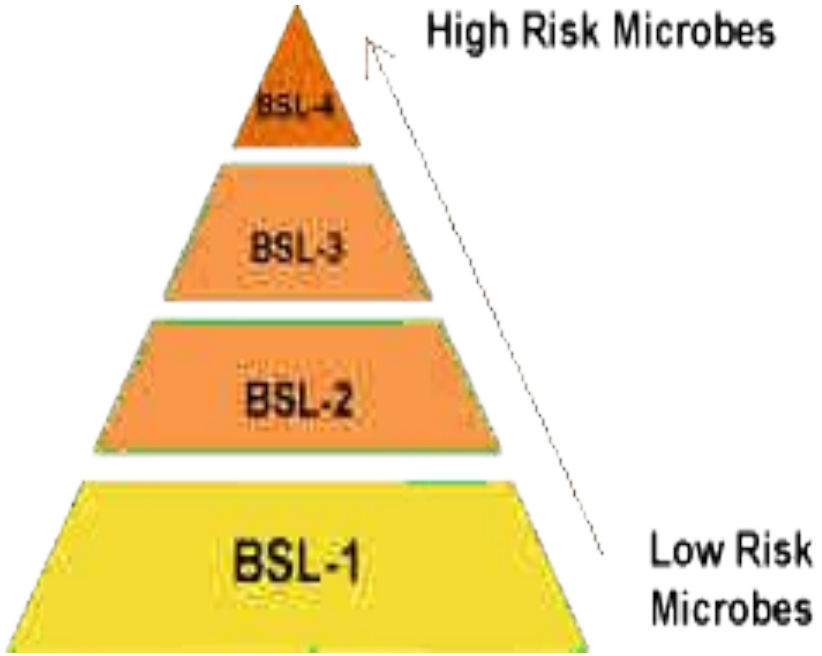
- Sharp objects like needles, slides, and blades
- Dry biohazardous wastes like excretions and contaminated cultures
- Liquid biohazardous wastes like human & animal blood, bodily elements, and blood elements

Biosafety = measures to prevent accidental release and/or exposure.

Biosecurity = measures to prevent intentional release and/or exposure.

BIOSAFETY LEVELs (BSL)

BSL-1	BSL-2	BSL-3	BSL-4
Lowest safety level Not known to cause disease in adult human Non-pathogenic microbe	Moderate danger if inhale, swallow or expose to skin Influenza	Severe or potentially lethal disease HIV, H5N1 flu	Highest safety level Life threatening disease Ebola, SARs - CoV2



The Difference in Laboratory Biosafety Levels 1, 2, 3 & 4?

BSL-1

- Practices, safety equipment and facilities are appropriate for undergraduate and graduate work in teaching/research laboratories
- Like 1st year biology labs and labs working with biomaterials not known to cause disease in healthy adults
- Research taking place on benches without the use of special contaminant equipment
- Did not required to be isolated from surrounding facilities.

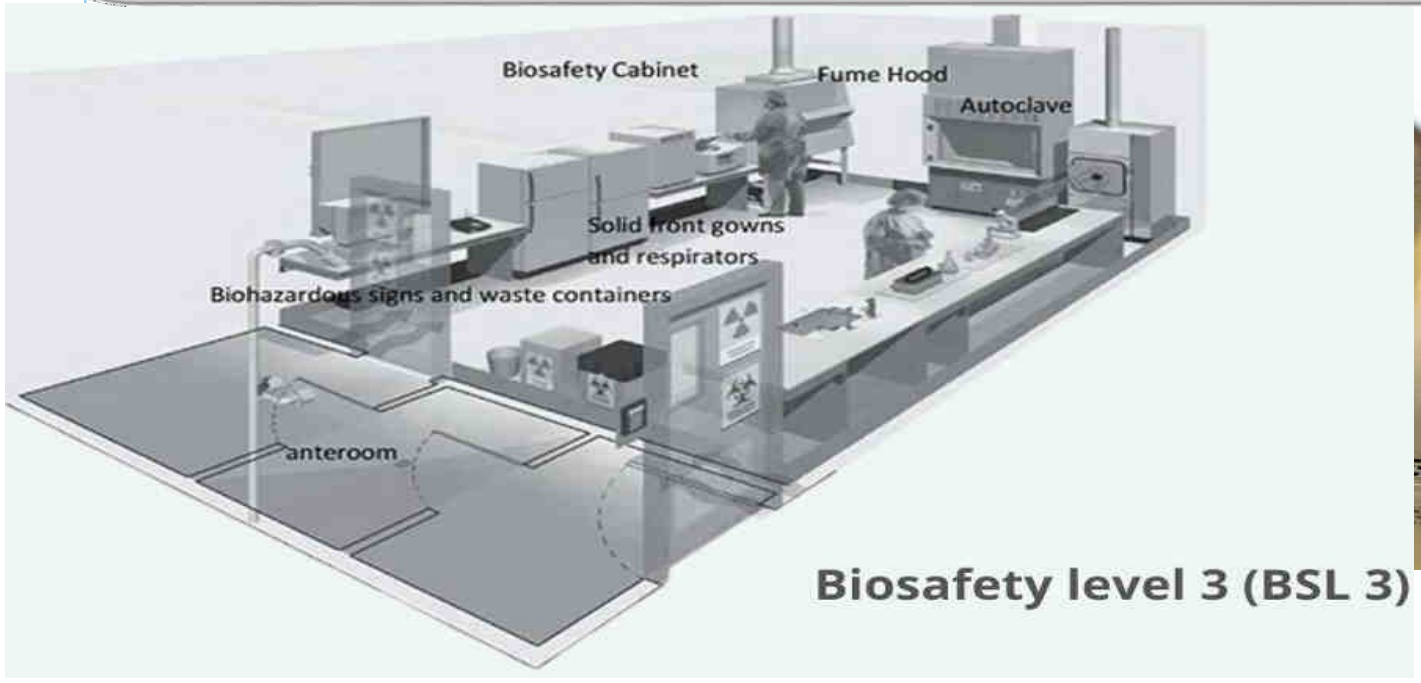
Biosafety Level 2 (BSL-2)

Practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community.

- BSL-2 builds upon BSL-1
- BSL-2 agents are associated with human disease of varying severity. (TB&HIV)
- REMEMBER!! Bringing certain agents in from environment will call for a BSL2 designation in order to propagate and contain in lab
- Lab equipment should be routinely decontaminated and must be after spills and before repair or removal

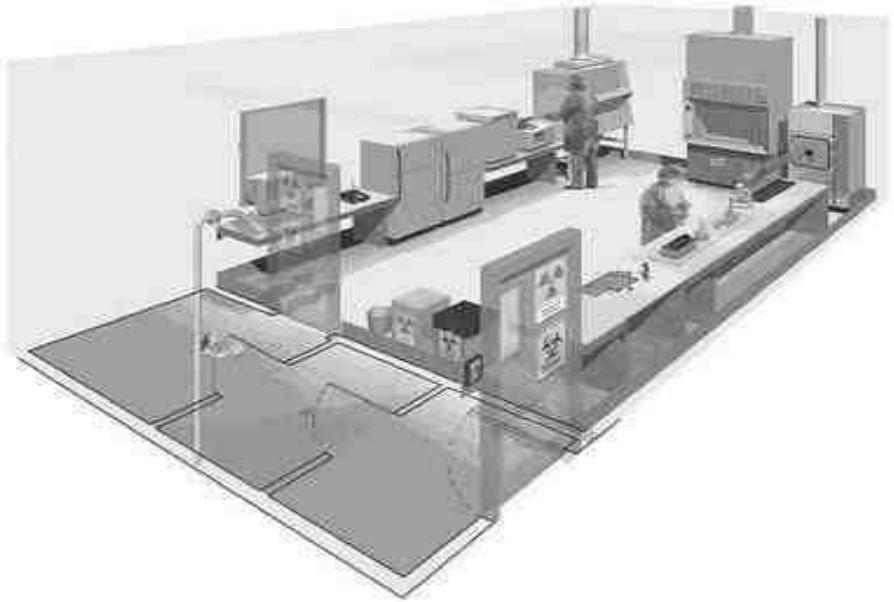
BSL-3 Facility design

- Practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents where the potential for infection by aerosols is real and the disease might have serious or lethal consequences.
- Additional Training is required for BSL3 work
- Consultation should be made with the Biosafety Officer before ordering, or working with any BSL3 agents



BSL-4 Facility design

- Entry must be limited by a secure, locked door. A means of documentation of persons entering and leaving the laboratory must be maintained.
- No one should work alone in the lab, a two-person rule should apply
- An inner disposable pair of gloves must be worn to prevent tears in the outer pair of gloves
- A method of communication for emergency contacts must be developed between the staff working in the laboratory
- A double-door, pass-through autoclave must be readily available for the materials passing out from Class III BSC



Biological Safety Cabinets (BSC's)

BSC's are typically Class II Type A cabinets which means:

- The air is cleaned as it goes into the hood to protect product
- Then the air is then pulled through the unit away from you for your protection
- Then finally it is HEPA filtered exhaust to protect you and the environment

•70% recirculated into hood
•30% exhausted into room

Biosafety in Virology Laboratory

All biosafety levels Personal Protective Equipment (PPE)

PPE provides a barrier between a route of exposure and the hazard

Protective clothing

- Lab coat
- Disposable latex or non-latex exam gloves

When torn or contaminated, remove gloves, wash hands, and put on new pair

PPE should NOT leave the work area

All biosafety levels Personal Protective Equipment (PPE)

People used to work in the clinical laboratory must use PPE, which includes gloves, gowns or laboratory coats, masks, goggles and face shields

Biosafety in Virology Laboratory

Disposal of biohazardous waste

PRACTICAL VIROLOGY

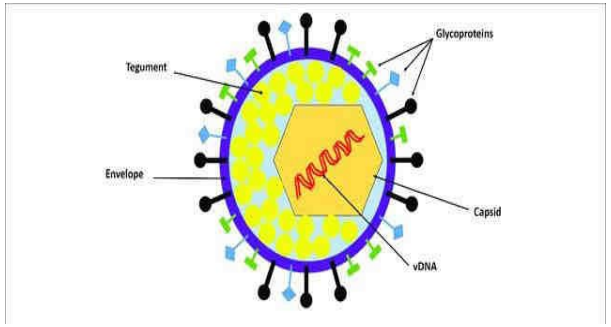
1-Definition of viruses

2-Virions

3-Shapes of viruses

4- Spreading of viruses

Virus: is a small infectious agent that replicates only inside the living cells of other organisms. Viruses can infect all types of life from animal and plants to microorganisms including bacteria and archaea. Viruses are found in almost every ecosystem on earth. The study of viruses is known's as virology a sub-specialty of microbiology.



What is Virion?

Virion: are virus practical that consist of two or three parts:

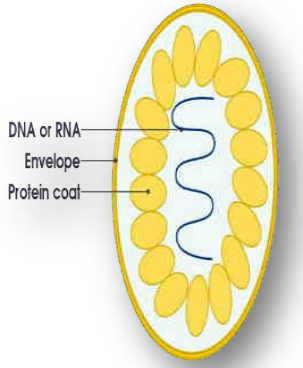
1. The genetic material made from either DNA or RNA (long molecule that carry genetic information)
2. A protein called capsid which surrounded and protect the genetic material.
3. An enveloped of lipids that surrounded the protein coat when they are outside the cell.

1-Simple helical

-has capsid shaped into filamentous or rod structure

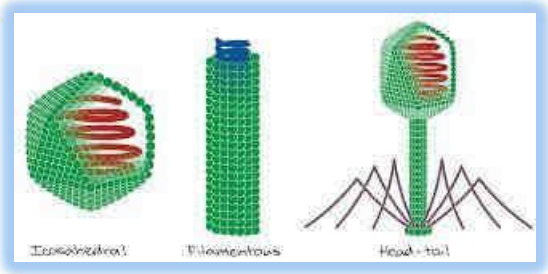
-have central cavity that enclosed its nucleic acid

-some these viruses are short while others are long



2 .-Icosahedral forms

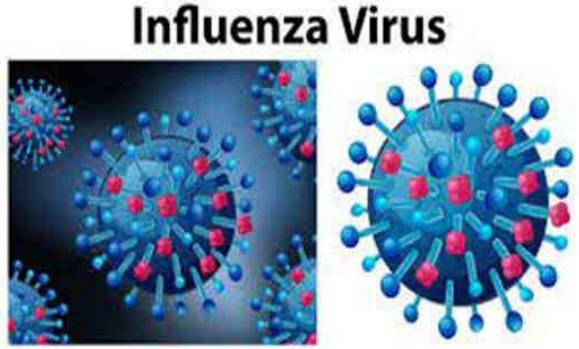
- Consisting of identical subunit that made up equilateral triangle
- Because the protein subunits that make up shapes are identical the virus doesn't have to waste a lot of its genome on encoding many different kinds of protein for its capsid. This lead to conservation of energy and genetic economy.
- The viruses are an important means of horizontal gene transfer which increase genetic diversity how that????? Because they carry the genetic material reproduce and involved through natural selection.



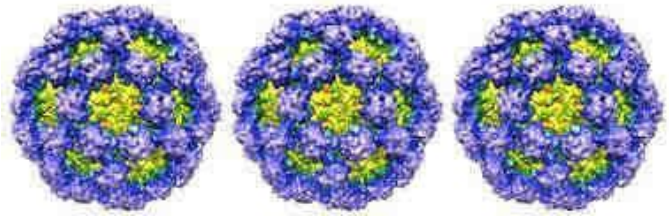
Icosahedral forms

Spreading of viruses

1. viruses in plants are often transmitted from plant by insects that feed on plant sap such as aphids.
2. viruses in animal can be carried by blood sucking these disease bearing organism are known as vectors Ex; influenza viruses are spread by coughing and sneezing.

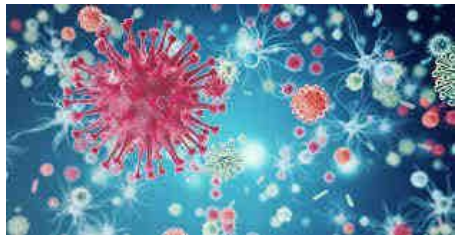


Ex: **Norovirus and rotavirus** common causes of viral gastroenteritis are transmitted by the fecal – oral route and are passed from person to person by contact entering the body in food or water.



Norovirus

Ex: **HIV** virus is one of several viruses transmitted through sexual contact and by exposure to infected blood



HIV

How can viruses treated medically?

By vaccines which confer an artificially acquired immunity to the specific viral infection

The establishment of a virology laboratory and diagnosis need to:

1. Physical infrastructure**:** is a Tanique that used in diagnostic virology laboratory contain

-Detection of viral antigens

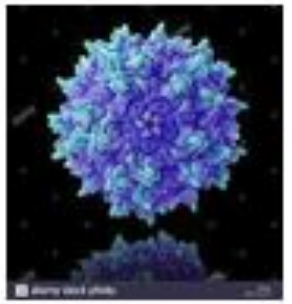
-Nucleic acid

-Antibiotic

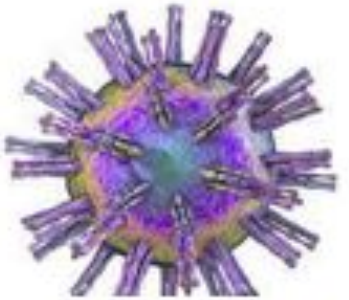
2. Human resources

3. Equipment and supplies

Biosafety Requirements



AAV



Herpes virus

Microorganisms can be divided into 4 groups (according to the risk posed to individual and community):-

group 1 : (no or low individual and community risk)

— That's mean that Microorganisms is unlikely to cause human or animal disease

— Ex… adino - associated virus (AAV)

(group 2):Moderate individual risk, low community risk

— A pathogen that can cause human or animal disease but is unlikely to be series hazards to :

— A…. Labrotary workers B…. Livestock C…. Environment

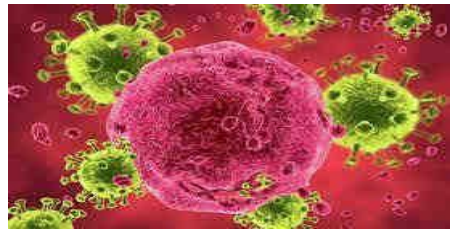
— Ex….. Herpes virus , FMD viruses

group 3 : (high individual risk , low community risk)

— A pathogen that usually causes series human or animal diseases

— but not ordinary spreads from infected individual to another

— Ex …. HIV , Rabies



HIV

group4: **(high individual and community risks**) — Pathogens that usually causes series human or animal disease and that can be readily transmitted from one individual to another direct or indirect methods.

Viral Identification

- Importance of identifying viruses in clinical and research settings
- Challenges: small size, need for host cells, diversity of viral genomes

Classical Methods

1-Cell culture techniques :-growth of viruses in cell lines to observe cytopathic effects.

2-Cytopathic effects (CPE) changes in host cells (lysis, syncytia formation).

3-Hemagglutination assays :- some viruses agglutinate red blood cells (influenza, measles).

Serological Methods:-

ELISA (Enzyme-Linked Immunosorbent Assay) :-detects viral antigens or antibodies in patient samples

Western blotting :-used in HIV confirmation testing

Neutralization tests :-measure the ability of antibodies to block viral infection.

Molecular Methods

1-Polymerase Chain Reaction (PCR, RT-PCR, qPCR):-

PCR: amplifies DNA;

RT-PCR use of RNA as a template. An additional step allows the detection and amplification of RNA. The RNA is reverse transcribed into complementary DNA (cDNA), using reverse transcriptase. viruses like SARS-CoV-2.

qPCR: quantifies viral load, important in monitoring HIV patients.

2- Next-Generation Sequencing (NGS):-is a rapid, high-throughput technology for massively parallel sequencing of DNA or RNA fragments, allowing for the simultaneous analysis of millions of DNA strands. It enables the study of entire genomes or targeted regions, providing detailed insights into genetic variation, gene expression, and epigenetic modifications.

Electron Microscopy

- Direct visualization of viral particles
- Provides structural information
- Used for rare and novel viruses

Any questions?