

Virology Lab Equipment & Safety

Lecture aims:

- Review the approaches to viral identification
- Explore viral isolation techniques
- Comprehending biosafety measurements

Laboratory safety

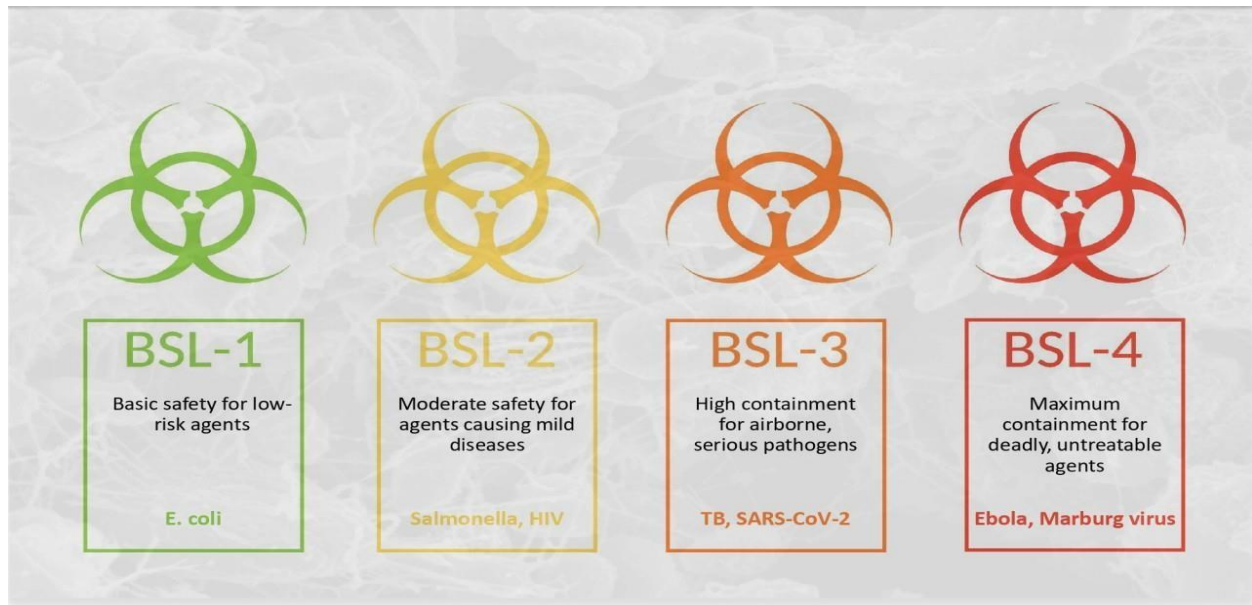
Laboratory safety involves a set of rules and procedures designed to prevent accidents and injuries in a lab setting.

Types of hazards in a lab

- Biological hazard: such as Hepatitis B, Hepatitis C, HIV.
- Chemical hazard: such as toxic liquids and fumes.
- Physical hazard: like fire and radiation.

Safety equipment

- 1- Gloves
- 2- Safety glasses/Goggles
- 3- Lab coats (should be fluid resistant)
- 4- Face shields
- 5- Gowns
- 6- Hazmat suit



Viral Isolation & Culture

Sample collection

Collection of a sample depends on two main aspects:

- **The type of viral infection.** □
- The test required.**

Proper sampling technique is essential to avoid potential **pre-analytical errors**.

Four considerations should be taken in Samples collection:

1. Sample from virus infected patient should be collected early in the course of the disease when maximum amount of virus is expected, and before any treatment and production of antibodies.
2. Correctly labeled (name, age, sex, date, type of specimen).
3. Collect the specimen from the appropriate site, an adequate amount of specimen, in appropriate tubes to maintain the integrity of the sample.
4. Storage of sample at appropriate temperatures (usually 4°C).

Viral Isolation

Viral isolation is the process of detecting and purifying a virus from a clinical or environmental sample by cultivating it in living cells.

1. Specimen Collection

- **Source:** Blood, respiratory secretions, stool, cerebrospinal fluid, tissue biopsies, etc.
- **Timing:** Ideally during the acute phase of infection when viral load is highest.
- **Transport:** Viral transport media (VTM) to preserve viability; which keep samples cold (2–8°C).

2. Transportation of Sample

Viral transport medium (VTM) contains proteins (albumin or gelatin) and antimicrobial agents in a buffered salt solution (adequate pH).

- Prevents specimen drying, maintains viral viability.
- Retards the growth of microbial contaminants.

3. Inactivation of Contaminants

- **Antibiotics:** Added to prevent bacterial growth in cultures.
- **UV or Heat Treatment:** Sometimes used to inactivate non-viral contaminants (though this risks damaging the virus itself).

4. Sample Preparation

- **Homogenization:** For solid tissues, mechanical disruption or enzymatic digestion to release viral particles.
- **Clarification:** Low-speed centrifugation is used to remove debris and cells.
- **Filtration:** Applying membrane filters (0.22–0.45 µm) to eliminate bacteria and larger particles while allowing viruses to pass.

5. Concentration (Optional but Useful)

- **Ultracentrifugation:** High-speed spin to pellet viruses.
- **PEG Precipitation:** Polyethylene glycol can precipitate viruses from solution. □
- **Dialysis:** Removes unwanted solutes while retaining viral particles.

Viral Culture

1- Cell line culture

- **Primary cell cultures:** are cells directly taken from a living organism
- **Diploid cell cultures:** are cell lines that are derived from primary cells but are more carefully managed to maintain healthy environment with limited passages.

- **Continuous cell cultures:** are immortal cell lines that can proliferate indefinitely under suitable conditions.
- **Organotypic cell cultures:** three-dimensional cell models that replicate the architecture and function of actual tissues.

2- Animal Inoculation

3- Embryonated Egg Inoculation

Viral DNA/RNA extraction & PCR

Lecture aims:

- Grasping viral DNA/RNA extraction approaches
- Knowing the difference between DNA and RNA extraction
- Exploring the techniques for PCR application

1. Sample Collection

- Biological samples (blood, saliva, swabs, or tissue homogenates) are collected.
- Proper storage and handling are essential to prevent degradation of viral DNA. **2. Virus Lysis**
- Viral particles are broken open to release their genetic material.
- This is achieved using **detergents, chaotropic salts, or enzymatic treatments** that disrupt the viral protein coat and lipid envelope.

3. Removal of Proteins and Contaminants

- Proteins are degraded using **proteinase K** or similar enzymes.
- Contaminants such as lipids and cellular debris are separated, often by centrifugation or chemical precipitation.

4. DNA Binding

- Released DNA is captured by binding to a solid phase (e.g., silica membrane or magnetic beads).
- Binding occurs in the presence of high concentrations of chaotropic salts, which promote nucleic acid affinity to the matrix.

5. Washing • Bound DNA solution is washed with ethanol-based or buffer

solutions.

- This step removes residual proteins, salts, and inhibitors that could interfere with downstream applications.

6. Elution

- Pure DNA is released from the binding matrix using a low-salt buffer or water.
- The eluted DNA is now ready for use in PCR, sequencing, or other molecular assays.

7. Quality Check

- DNA yield and purity are assessed using spectrophotometry (e.g., absorbance at 260/280 nm).
- Integrity can be checked by gel electrophoresis

Viral DNA vs Viral RNA Extraction Comparison

1. Purpose

DNA Extraction: Isolate viral DNA (stable target).

RNA Extraction: Isolate viral RNA (highly unstable, easily degraded).

2. Sample Handling

DNA: Low degradation risk; standard precautions.

RNA: High degradation risk; strict RNase-free technique required.

3. Lysis & Extraction Chemistry

DNA Extraction

- Detergents + proteinase K for capsid/envelope disruption.
- DNA binds silica under chaotropic salts.
- DNA tolerates harsh chemical conditions. **RNA Extraction**
- Must include RNase inhibitors.
- Uses TRIzol or silica with guanidinium thiocyanate.
- Reducing agents (β-mercaptoethanol/DTT) to inactivate RNases.

RNase inhibitors: Bind to RNases and block their activity to protect RNA from degradation.

TRIzol (phenol + guanidinium thiocyanate): Immediately lyses cells and denatures proteins, especially RNases, preserving RNA.

Silica + guanidinium thiocyanate: Chaotropic salt forces RNA to bind to silica membranes so impurities can be washed away.

β-mercaptoethanol (BME): Strong reducing agent that breaks disulfide bonds in RNases, inactivating them.

DTT (dithiothreitol): Another reducing agent that disrupts RNase structure by reducing disulfide bonds, preventing RNA degradation.

4. Purification Methods

DNA: Phenol-chloroform or silica. Rarely needs RNase.

RNA: TRIzol or silica; often requires DNase treatment.

5. Yield & Quality Control

DNA: Nano drop 260/280 » 1.8 in measurement; stable, stored at -20°C.

RNA: Nano drop 260/280 » 2.0 in measurement; must be stored at -80°C; avoiding freeze– thaw.

6. Applications

DNA: PCR, sequencing, diagnostics for DNA viruses.

RNA: RT-PCR, RNA-seq, diagnostics for RNA viruses.

Polymerase Chain Reaction (PCR)

is a lab method that makes millions of copies of a specific DNA piece so it can be detected or studied.

1. **Mix** — put template DNA, two short **primers**, free nucleotides (dNTPs), heat-stable **DNA polymerase**, and buffer in a tube.
2. **Denature (heat)** — heat to ~95°C to unzip the double helix into single strands.
3. **Anneal (cool)** — cool to ~50–65°C so primers stick (bind) to the matching places on each single strand.
4. **Extend (warm)** — warm to ~72°C; polymerase adds dNTPs onto primers, making new DNA strands.
5. **Repeat cycles** — repeat steps 2–4 for ~25–40 cycles; each cycle doubles the target DNA amount (exponential gain).
6. **Final hold** — a final extension ensures incomplete strands finish; then store the product for analysis.

PCR application for viral DNA and RNA

Viral DNA PCR

- Starts with **viral DNA** directly.
- Uses **regular PCR** (denaturation → annealing → extension).
- No need for reverse transcription.
- Target is **DNA viruses** (e.g., HSV, Adenovirus).

Viral RNA PCR

- Starts with **viral RNA**, which cannot be amplified directly.
- First step is **reverse transcription (RT)** to convert RNA → cDNA.
- Then regular PCR is performed.

- Used for **RNA viruses** (e.g., SARS-CoV-2, Influenza).

If you want an ultrashort one-line version, I can provide that too.

Main Differences

- RNA extraction is more delicate.
- Buffers contain RNase inhibitors for RNA work.
- DNA is easier and more chemically robust.
- RNA protocols risk DNA contamination, which may require DNase step.
- RNA extraction usually performed on ice.

Review questions

- 1. What is the main purpose of viral DNA or RNA extraction? Answer:** To isolate the viral genetic material so it can be detected or analyzed.
- 2. Why is RNA extraction more delicate than DNA extraction? Answer:** Because RNA is easily degraded by RNases and requires strict RNase-free handling.
- 3. What role does proteinase K play during viral lysis? Answer:** It breaks down viral and cellular proteins to release nucleic acids.
- 4. Why are RNase inhibitors needed in RNA extraction? Answer:** They block RNases to protect RNA from degradation.
- 5. Which chemical method is commonly used for RNA extraction—TRIZOL or silica columns? Answer:** Both are used, but TRIZOL is one of the most common methods.
- 6. What does PCR do to a specific piece of DNA? Answer:** It makes millions of copies of that DNA segment.
- 7. What is the purpose of the denaturation step in PCR? Answer:** To heat the DNA and separate it into single strands.
- 8. Why is reverse transcription needed before PCR when working with viral RNA? Answer:** Because RNA must first be converted into complementary DNA (cDNA) for PCR to work.

9. What is one application of viral DNA PCR? Answer: Detecting DNA viruses such as HSV or Adenovirus.

10. What does a 260/280 ratio of about 2.0 indicate in RNA extraction?

Answer: It indicates that the RNA is pure and high quality.

ELISA Tests for Viral Identification

Lecture aims:

- Understand the Principles of ELISA
- Differentiate Between ELISA Formats ● Explore ELISA Applications in Virology

What is ELISA?

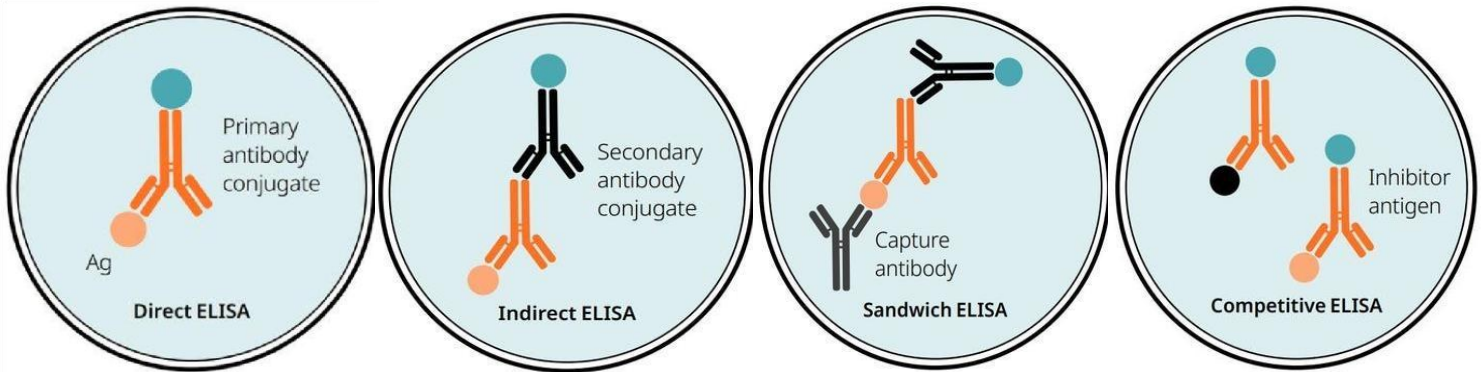
ELISA (Enzyme-Linked Immunosorbent Assay) is a biochemical technique used to detect and quantify the presence of specific molecules typically proteins such as antibodies or antigens in a liquid sample.

How does ELISA work?

- It works by:
1. Binding the target molecule to a solid surface (usually a microplate).
 2. Using an enzyme-linked antibody that specifically binds to the target.
 3. Adding a substrate that the enzyme converts into a detectable signal, usually a color change.

Types of ELISA:

- 1- **Direct ELISA:** Detects antigens directly using an enzyme-linked primary antibody. Simple but less sensitive.
- 2- **Indirect ELISA:** Measures antibodies in a sample by using a secondary enzyme-linked antibody for detection. It's more sensitive.
- 3- **Sandwich ELISA:** Captures antigens between two antibodies. Highly sensitive for detecting low-abundance proteins.
- 4- **Competitive ELISA:** Measures antigen or antibody by competition with a known concentration. Used when targets are small or scarce.



The choice of ELISA type depends on:

1. The stage of infection
2. The virus type
3. The purpose (diagnosis, surveillance, or vaccine monitoring)

According to the stage of infection

Infection Stage	Immune Response Status	Recommended ELISA Type	Target Detected	Purpose
Early (Acute Phase)	Virus actively replicating	Direct or Sandwich ELISA	Viral antigens	Detect presence of virus
Mid-phase	Initial antibody production	Indirect ELISA	IgM antibodies	Confirm recent infection
Late or Recovery	Established immune response	Indirect ELISA	IgG antibodies	Confirm past exposure
Postvaccination	Immune memory formation	Indirect or Competitive ELISA	IgG antibodies	Assess vaccine response

According to virus type

1. Indirect ELISA detects antibodies against viruses
 - **Used for:** HIV, Rubella, Hepatitis C.
 - **Why:** These viruses trigger strong antibody responses. Indirect ELISA is ideal for screening patient serum for virus-specific antibodies. □ **Example:** HIV antibody tests use indirect ELISA to detect anti-HIV antibodies in blood.
2. Direct ELISA detects viral antigens directly
 - **Used for:** Detecting viral proteins in samples.

- **Why:** Useful when the virus itself (or its proteins) is present in high concentration.
- **Example:** Some rapid tests for viral antigens (like influenza or COVID-19) use direct ELISA formats.

3. Sandwich ELISA highly sensitive antigen detection

- **Used for:** Hepatitis B surface antigen (HBsAg), Dengue virus NS1 protein.
- **Why:** Ideal for detecting low-abundance viral antigens with high specificity.
- **Example:** Dengue NS1 antigen tests use sandwich ELISA to capture and detect the viral protein.

4. Competitive ELISA detects small viral antigens or antibodies

- **Used for:** Situations where the antigen is small or has only one epitope.
- **Why:** Useful when standard sandwich formats aren't feasible. □ **Example:** Some tests for viral toxins or small viral proteins use competitive ELISA.

Review questions

1-What are the key differences between Direct and Indirect ELISA?

Direct ELISA uses one labeled antibody; Indirect ELISA might use two or more, increasing sensitivity.

2-How does the Sandwich ELISA improve specificity compared to other formats? It uses two antibodies targeting different epitopes of the same antigen.

3-Why is it important to include a standard curve in quantitative ELISA assays? It allows accurate calculation of unknown concentrations from absorbance values.

4-In what scenarios would Competitive ELISA be preferred over other types? When detecting small molecules or antigens with only one epitope.

5-How can ELISA be used to detect past versus current viral infections? By measuring IgG for past and IgM for recent or active infections.

6-What factors can affect the sensitivity and specificity of an ELISA test? Antibody quality, incubation time, washing steps, and cross-reactivity.

7-If an ELISA test shows high absorbance but the patient is asymptomatic, what could be the possible explanations?

Past infection, false positive, or subclinical immune response.

8-How would you design an ELISA to detect antibodies against a newly discovered virus? Coat the plate with viral antigen and use patient serum as the primary antibody.

9-What are the limitations of ELISA in viral diagnostics, and how can they be addressed? Cross-reactivity and low sensitivity; improved antibodies and confirmatory tests help.

10-Can an antibody act as both a capture and detection molecule in ELISA? Explain with an example.

Yes, if it's bi-specific or labeled differently, like in some monoclonal setups.

Electron Microscope for Virus Identification

Lecture aims:

- Differentiating between electron microscope types.
- Revealing the uses of electron microscope in virology.
- Discovering the anatomy of electron microscope.

What is an electron microscope (EM)?

An electron microscope (EM) is a microscopy instrument that employs a beam of accelerated electrons as the illumination source, rather than visible light, to achieve resolutions down to ~0.1 nm over 1,000 times superior to optical microscopes.

Transmission EM (TEM): Electrons transmit through ultrathin specimens (~50-100 nm) for internal structure visualization.

Scanning EM (SEM): scans surfaces for 3D topography at ~1-10 nm resolution.

Why electrons instead of photons?

Visible light in optical microscopes has wavelengths spanning roughly 400–700 nm, setting a resolution limit where finer details blur due to diffraction. In contrast, accelerated electrons in electron microscopes exhibit de Broglie wavelengths of about 0.002–0.005 nm (One picometer (pm) is equal to 0.001 nanometers (nm), meaning 1 nm equals 1000 pm. To convert picometers to nanometers, multiply the number of picometers by 0.001; for example, 15 pm is 0.015 nm), yielding a wavelength ratio of approximately 100,000:1 (light much longer). This dramatic reduction enables electron microscopes to resolve structures down to atomic scales, far surpassing light microscopes for nanoscale imaging.

What are the uses of EM in virology?

Electron microscopy (EM) in virology enables direct visualization of virus particles at nanometer resolution, allowing identification by morphology, size, and structure essential since viruses are too small (~20–300 nm) for light microscopy.

How to prepare specimens for EM?

Preparing viral specimens for electron microscopy involves distinct steps for TEM and SEM. For **TEM**, viruses are isolated from samples (e.g., via centrifugation), applied as a thin droplet to a carbon-

coated grid, negatively stained with heavy metals like phosphotungstic acid for contrast, blotted dry, and air-dried before imaging thin, translucent particles. For **SEM**, samples are fixed chemically (e.g., glutaraldehyde), dehydrated through graded alcohol series, critical-point dried or sputter-coated with gold for conductivity, and mounted to visualize surface topography.

Both require sterile handling in a vacuum-compatible setup, with TEM suiting internal ultrastructure and SEM for 3D morphology.

Anatomy of TEM (Transmission Electron Microscope)

TEM images internal structures by transmitting electrons through ultra-thin specimens. Components in vertical order from top to bottom:

- Electron Gun:** Emits electrons via heated filament (top of column).
- Condenser Lens System:** Focuses electron beam into a narrow probe.
- Specimen Stage:** Holds thin sample (~50-100 nm thick) for beam passage.
- Objective Lens:** Forms first magnified image of transmitted electrons.
- Intermediate/Projector Lenses:** Further magnify and project the image.
- Fluorescent Screen/CCD Camera:** Displays or records the final image (bottom).

Anatomy of SEM (Scanning Electron Microscope)

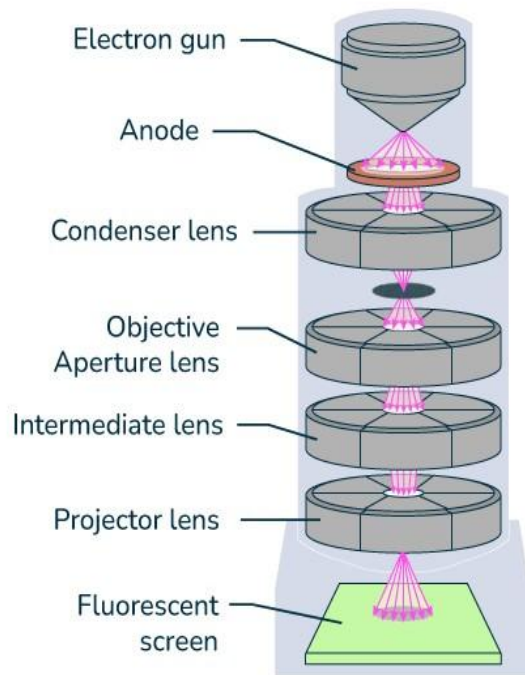
SEM scans surfaces for 3D topography using reflected/scattered electrons. Components from top to bottom:

- Electron Gun:** Generates fine electron beam.
- Condenser Lenses:** Focuses beam to ~1-10 nm spot.
- Scanning Coils:** Raster-scans beam across specimen surface.
- Specimen Chamber/Stage:** Holds bulk sample under vacuum.
- Detectors (SE/BSE):** Captures secondary/backscattered electrons for signal.
- Image Processor/Display:** Builds image from scanned signals.

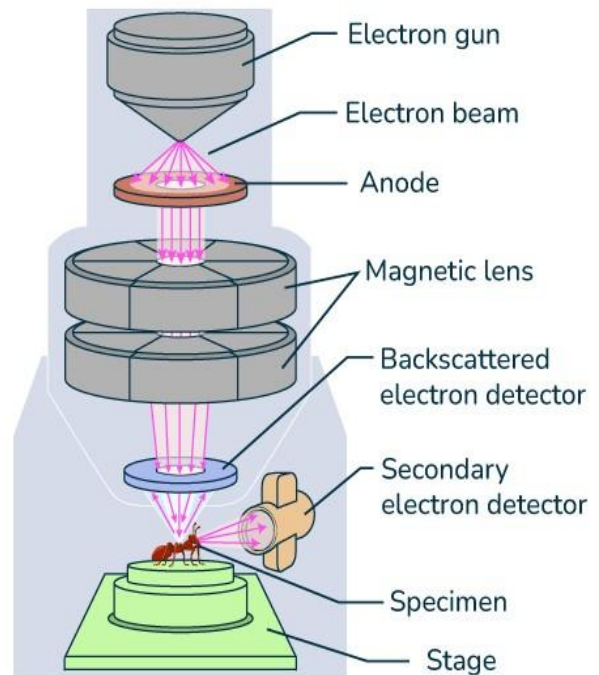
Feature	SEM	TEM
Full Name	Scanning Electron Microscope	Transmission Electron Microscope
Type of Image	3D surface image	2D internal structure image

How It Works	Electron beam scans the specimen surface and detects scattered/secondary electrons	Electron beam passes through an ultrathin specimen and detects transmitted electrons
Resolution	Lower (1–10 nm)	Higher (0.1–1 nm)
Magnification	Up to ~200,000×	Up to ~1,000,000×
Specimen Thickness	Thick specimens allowed (no sectioning needed)	Very thin sections (50–100 nm) required
Type of Information	Surface topology, texture, morphology	Internal ultrastructure, organelles, viral cores, crystals
Sample Preparation	Easier; coating with gold/palladium often needed	Complex; fixation, dehydration, resin embedding, ultrathin sectioning
Vacuum Requirement	High vacuum; some instruments allow variable pressure	High vacuum always required
Detectors Used	Secondary electron detector, backscattered electron detector	Transmitted electron detector, CCD cameras
Electron Path	Beam scans <i>across</i> surface	Beam goes through specimen
Use in Virology	Virus morphology, surface details, particle distribution on cells	Internal virus structure, capsid symmetry, nucleic acid cores
Image Appearance	3D-like, dramatic shadows	Flat but extremely detailed internal structures
Cost & Complexity	Less expensive, easier to operate	More expensive, requires expert handling

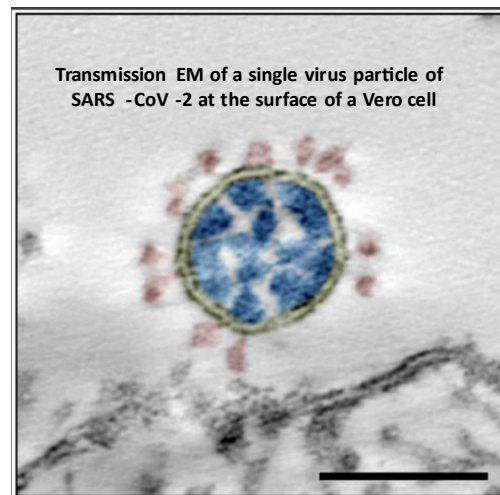
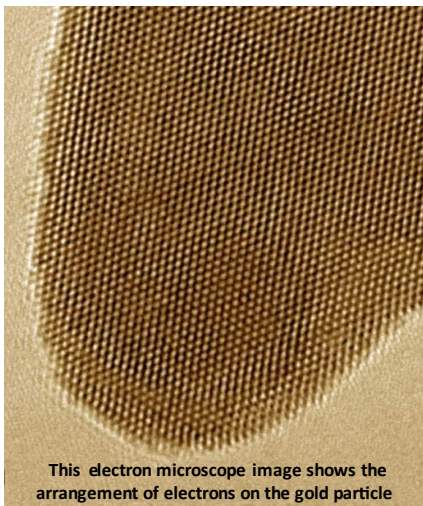
Diagram of Electron Microscope



Transmission Electron Microscope (TEM)



Scanning Electron Microscope (SEM)



Review questions

1-What is the main advantage of electron microscopes over light microscopes?

Answer: Electron microscopes have much shorter wavelengths, allowing higher resolution and magnification.

2-What type of electron microscope is best for viewing the internal structure of viruses?

Answer: Transmission Electron Microscope (TEM).

3-Which electron microscope is used to study the surface of virus particles in 3D?

Answer: Scanning Electron Microscope (SEM).

4-Why must viral specimens be placed in a vacuum inside an electron microscope?

Answer: To prevent electrons from scattering in air and ensure a clear beam.

5-What stain is commonly used for negative staining of viruses for TEM imaging?

Answer: Heavy metal stains like phosphotungstic acid.

6-How does electron acceleration affect the wavelength of electrons used in microscopy?

Answer: Higher acceleration increases momentum and decreases electron wavelength.

7-What is one key use of electron microscopy in virology?

Answer: Identification and visualization of virus particles.

8-When is electron microscopy especially useful in virology diagnostics?

Answer: For detecting unknown or emerging viruses when other tests fail.

9-What kind of images does SEM produce?

Answer: 3D surface topography images of specimens.

10-Why must specimens for SEM be coated with a conductive material like gold?

Answer: To prevent charging and improve image quality.