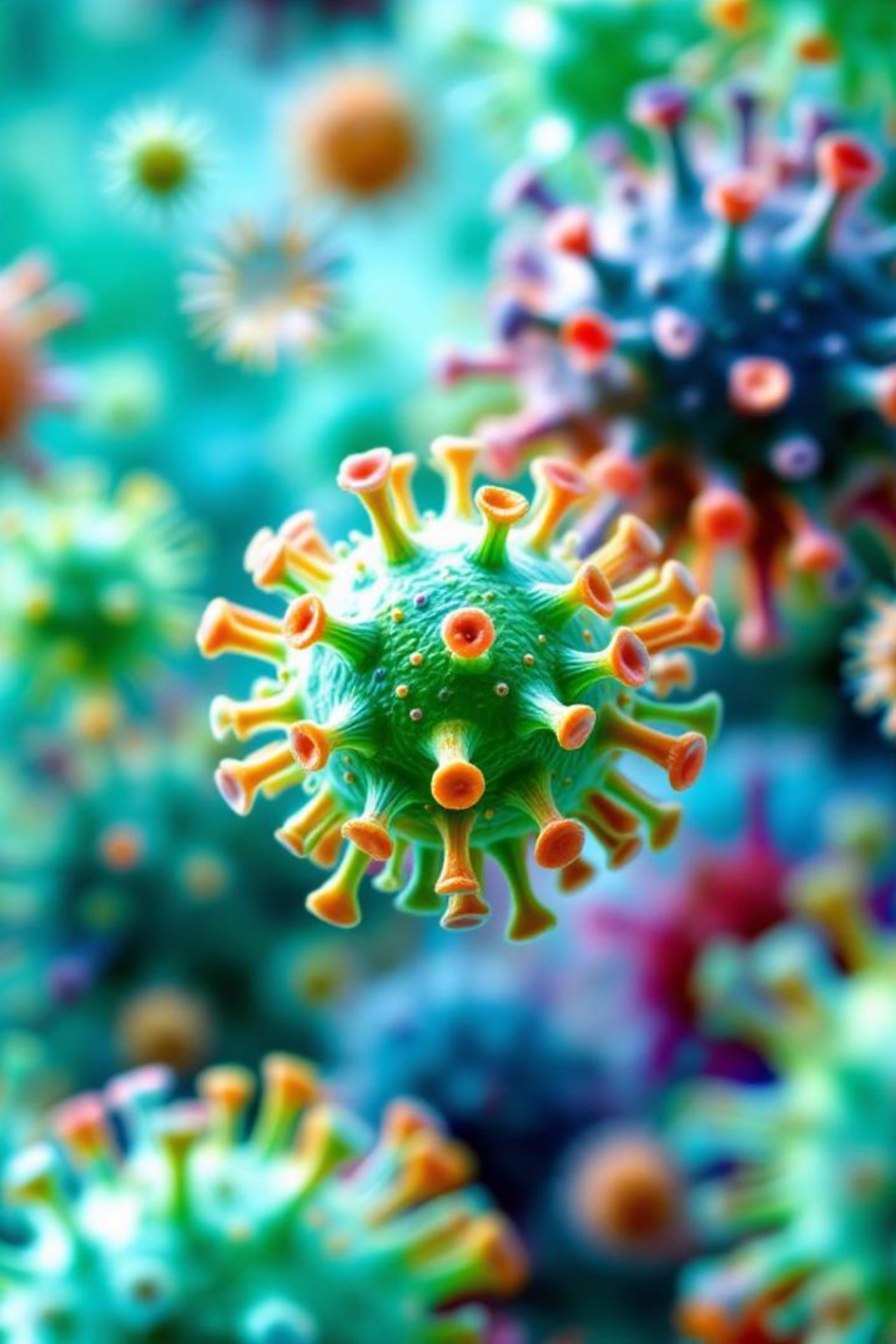


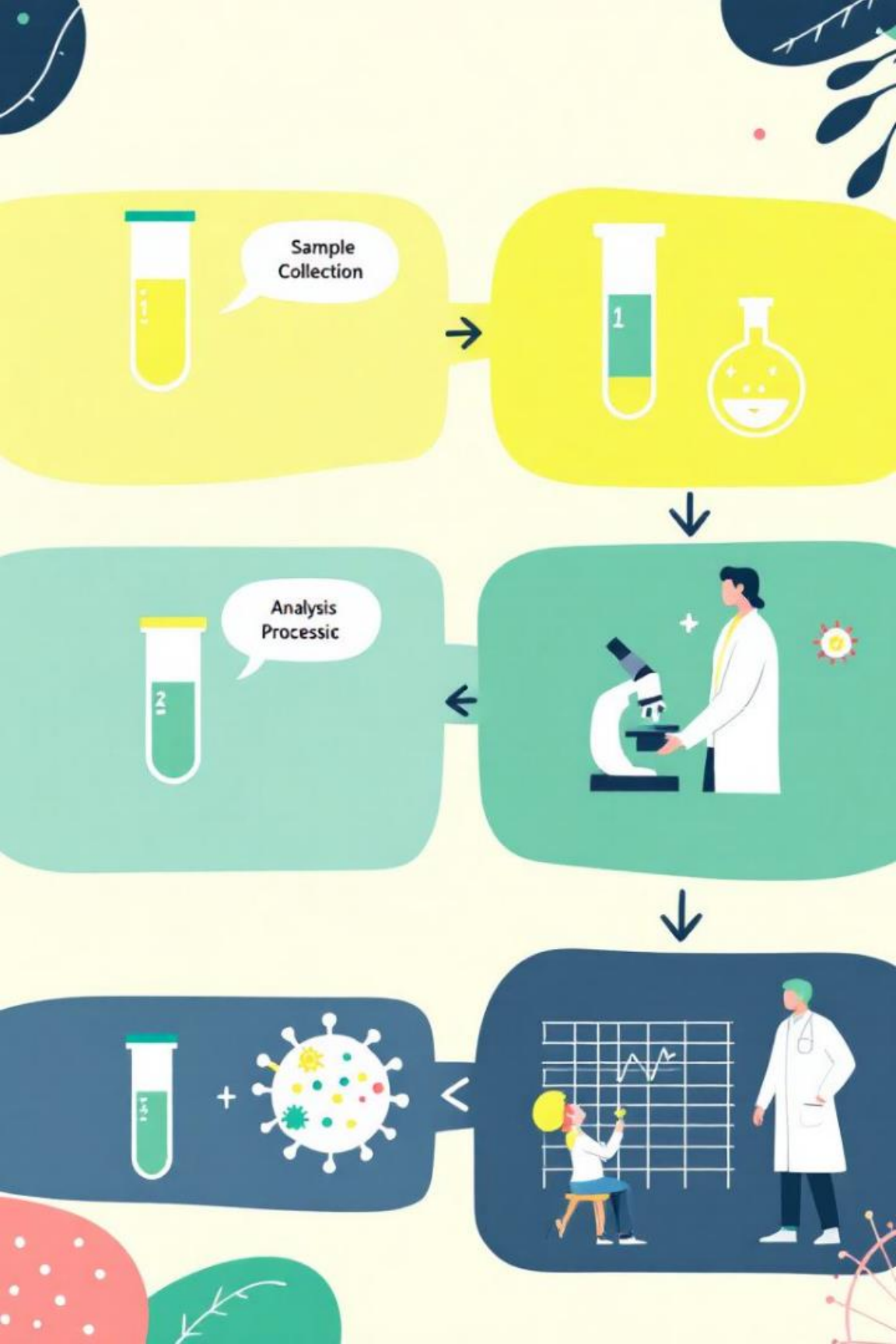


Diagnostic Virology

By:
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Welcome to the world of diagnostic virology! This presentation, prepared by Assistant Lecturer Zahraa A. Sahan, will guide you through the essential techniques used to identify and characterize viral infections. We will explore the methods for sample collection, detection of cytopathic effects, direct identification of virions, viral antigens, and nucleic acids, as well as virus isolation and serological assays. Prepare to dive into the methodologies that form the cornerstone of modern virological diagnostics.



An Overview

1 Sample Collection

Proper specimen acquisition is the foundation of accurate diagnosis.

2 Cytopathic Effects

Observing cellular changes caused by viral infection.

3 Direct Detection

Identifying virions, antigens, and nucleic acids directly from samples.

4 Virus Isolation

Culturing viruses in cells to amplify and study them.

These are the core methodologies in diagnostic virology. Each method provides crucial insights into the presence, nature, and impact of viral infections.



Types of Viral Diagnosis

Direct Methods

Detect virus/viral components

- (antigens, nucleic acids).

Indirect Methods

Detect host immune response

- (antibodies)

Specimen Selection Based on Clinical Syndrome

Syndrome	Specimen
Respiratory	Nasal or throat swab; nasopharyngeal aspirate; sputum
Enteric	Feces
Genital	Genital swab, urine
Eye	Conjunctival (and/or corneal) swab
Skin	Vesicle fluid/swab/scraping; biopsy solid lesion
Central nervous system	Cerebrospinal fluid; feces (enteroviruses)
Any	Blood for serology ^b

Choosing the correct specimen is essential for accurate diagnosis. Different clinical syndromes require different samples to effectively detect the presence of a virus. Respiratory illnesses often involve nasal swabs or sputum, while enteric issues necessitate fecal samples. Understanding these relationships optimizes diagnostic success.





Guidelines for Effective Specimen Collection



Keep Cold

Maintain specimens cold and moist to preserve viral integrity.



Transport Medium

Use virus transport medium with protein and antibiotics.



Labeling

Clearly label with patient details and collection date.

Effective specimen collection hinges on maintaining the sample's integrity. Keeping the sample cold, using appropriate transport media containing proteins and antibiotics, and thoroughly labeling each sample are crucial steps. Additionally, provide a complete clinical history with each specimen to guide testing.

Essential Diagnostic Methods

The most effective diagnostic methods are characterized by speed, simplicity, sensitivity, specificity, and cost-effectiveness. ELISA and PCR technologies have revolutionized diagnostic virology due to their ability to rapidly and accurately detect viral infections. These methods are now the preferred choice for a wide range of clinical indications.



Speed

Rapid results are crucial.



Simplicity

Easy to perform and interpret.



Sensitivity

Detect even low viral loads.



Specificity

Accurately identify the virus.





Cytopathic Effects (CPE) in Viral Infections

Cell enlargement

Infected cells may undergo significant enlargement.

Syncytia formation

Fusion with adjacent cells to form syncytia.

Inclusion bodies

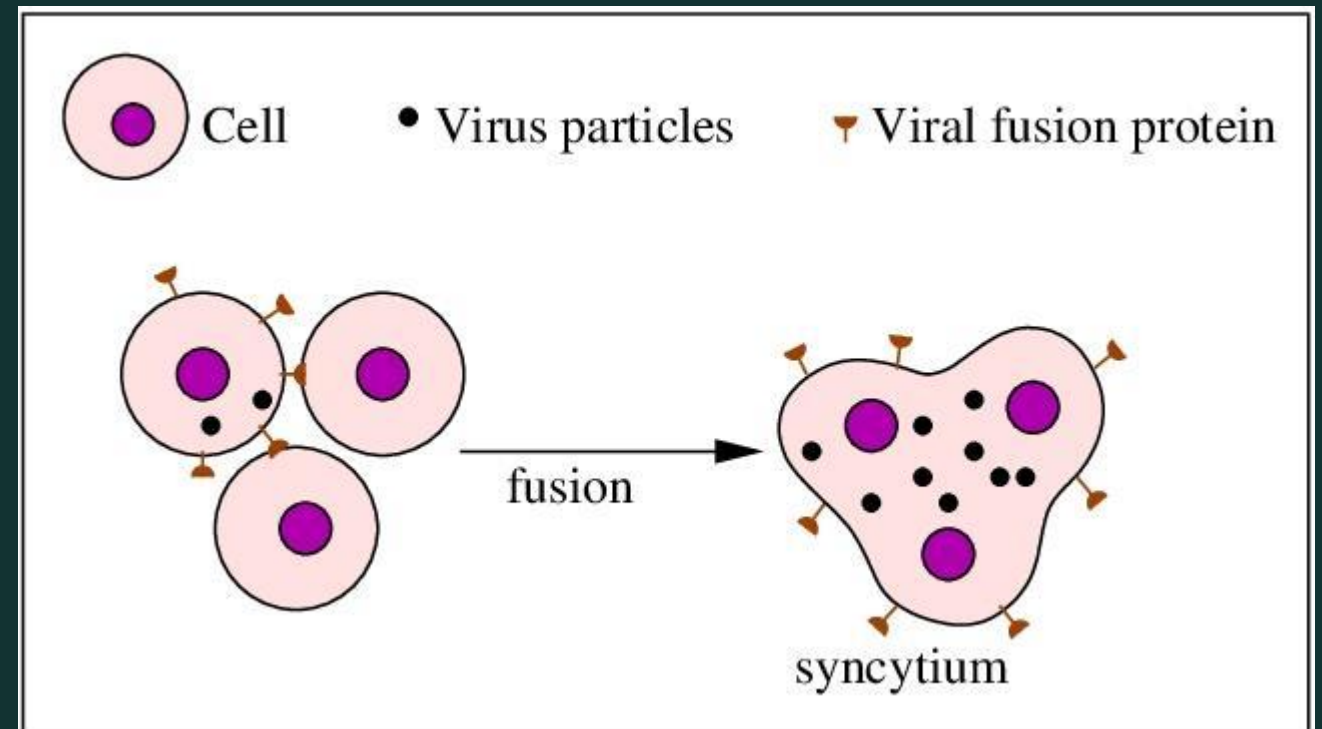
Appearance of nuclear or cytoplasmic inclusion bodies.

Cytopathic effects (CPE) refer to the morphological changes in cells caused by viral infection. Common examples include cell enlargement, fusion with adjacent cells to form syncytia, and the appearance of inclusion bodies. These effects can be observed under a light microscope.

INCLUSION BODIES



Inclusion bodies, sometimes called elementary bodies, are nuclear or cytoplasmic aggregates of stable substances, usually proteins. They typically represent sites of viral multiplication in a bacterium or a eukaryotic cell and usually consist of viral capsid proteins.





Direct Detection of Virions: Electron Microscopy

1

Sample Preparation

Prepare virus-containing fluid.

2

Grid Application

Place fluid on a grid for virion adherence.

3

Negative Staining

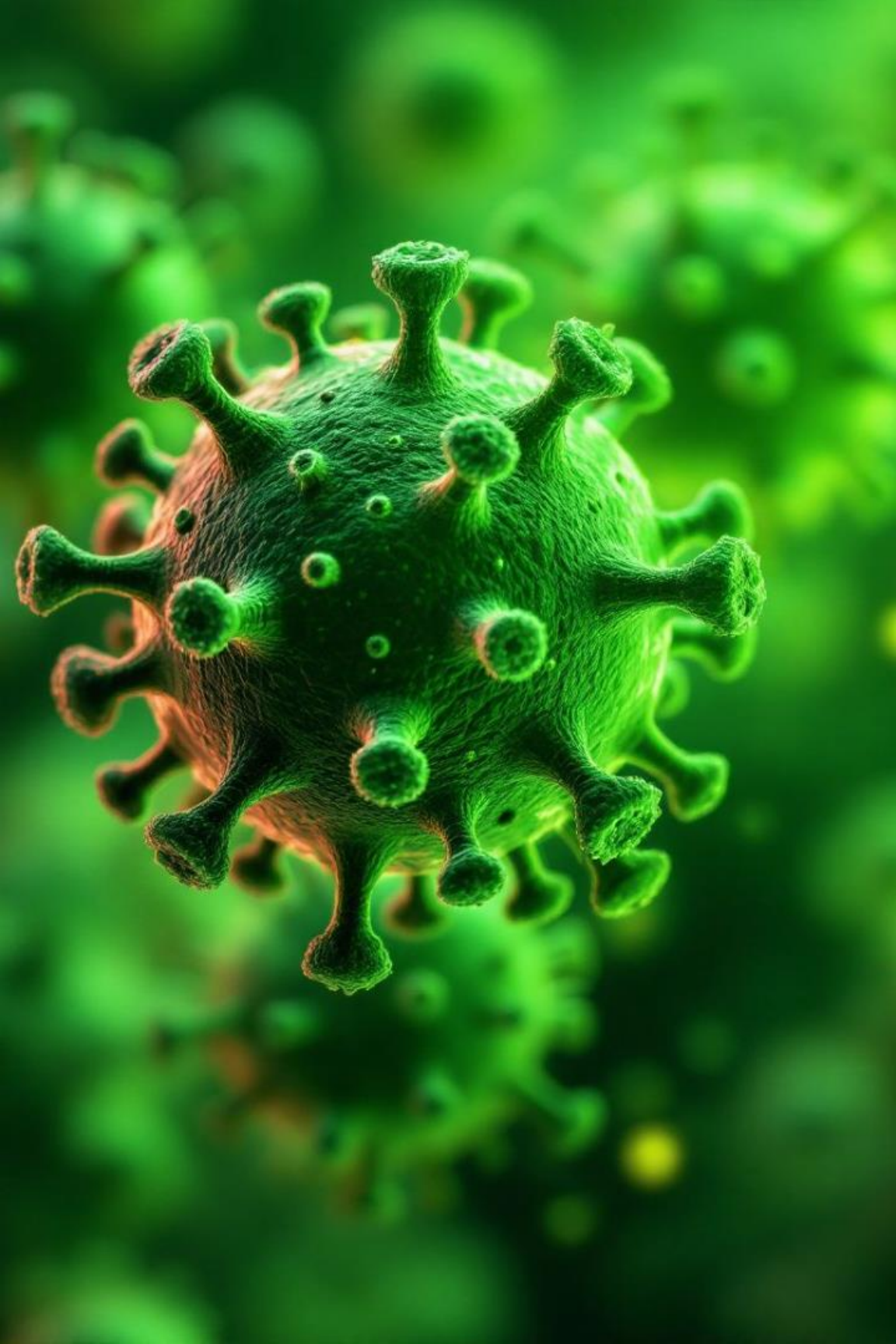
Apply negative stain for contrast.

4

Observation

Examine under electron microscope.

Electron microscopy was instrumental in discovering several new groups of previously non-cultivated viruses. The morphology of most viruses is sufficiently characteristic to allow assigning many viruses to the correct family by appearance. The most widely used procedure is negative staining, where virus-containing fluid is placed on a grid.



Electron Microscopy



Discovery Tool

Identified new viruses like rotavirus and calicivirus.



Morphology

Allows classification of viruses into families.

Electron microscopy played a pivotal role in discovering several previously unknown viruses in feces during the 1970s. This technique allows for direct visualization of virions, and the morphology of most viruses is sufficiently distinct to classify them into correct families. Negative staining enhances the visibility of viruses.



Limitations of Electron Microscopy in Diagnostics

Low Sensitivity

Requires high viral concentrations (at least 10^6 virions per mL).

Sample-Dependent

Virus levels often surpass this threshold in feces and vesicle fluid, but not in respiratory mucus.

Impractical for Batch Testing

Quick for one or several samples, but impractical for large batch testing.

The biggest limitation of electron microscopy as a diagnostic tool is its low sensitivity, requiring high viral loads for detection. While such levels are often surpassed in feces and vesicle fluid, they are less common in respiratory mucus. Although the method can be quick for a few samples, it is impractical for large-scale testing. Also it needs skilled personnel.

Detection of Viral Antigens

A variety of immunological assays, such as ELISA, latex particle agglutination, hemagglutination, and immunodiffusion, can be used to detect viral antigens in patient samples. These assays are highly specific and sensitive, allowing for rapid identification of viral infections. Each assay has its own advantages and limitations depending on the virus being targeted and the clinical context.

1

ELISA

Enzyme-linked immunosorbent assay.

2

Agglutination

Latex particle agglutination.

3

Hemagglutination

Detects viral hemagglutinins.



ELISA

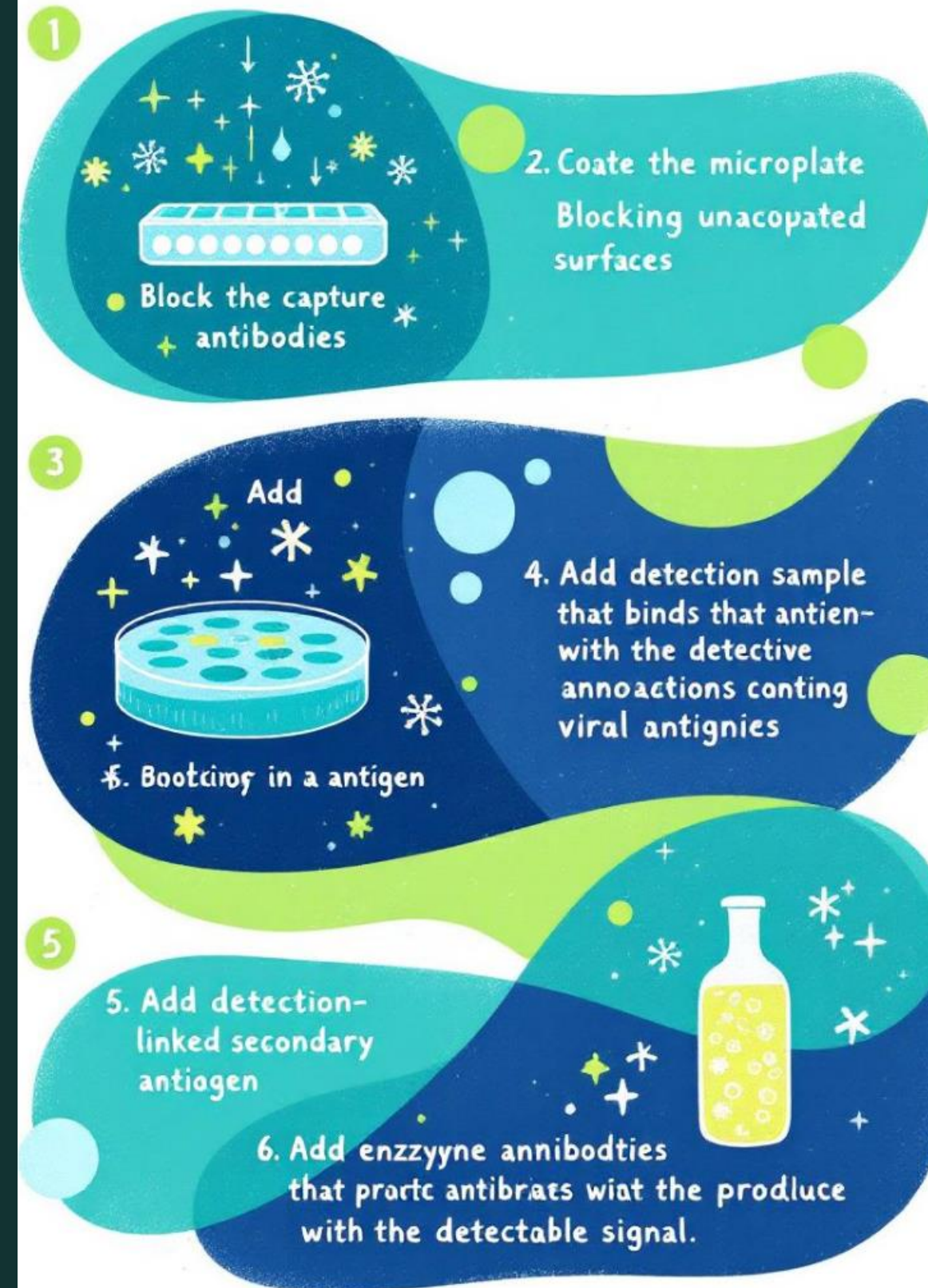
1ng

Minimum detectable viral antigen per milliliter.

The introduction of ELISA revolutionized diagnostic virology before the widespread use of PCR and remains essential for specific applications.

Highly sensitive, it can detect less than 1ng of viral antigen per milliliter in patient samples.

ELISA can be designed to detect either antigens or antibodies, making it a versatile tool in virology.





Latex Particle Agglutination

Latex particle agglutination is a simple immunoassay where latex beads coated with antiviral antibodies agglutinate in the presence of viral antigens. Results are read by eye within minutes. Diagnostic kits are popular with small labs and medical practitioners.

Simplicity

Easy to perform and interpret.

Speed

Results available within minutes.

Limitations

Low sensitivity and specificity may lead to false results.



Detection of Viral Nucleic Acids

Nucleic acid detection, particularly using polymerase chain reaction (PCR) assays, has become a cornerstone of viral diagnostics. Rapid advances in nucleic acid sequencing and comprehensive sequence databases have significantly enhanced the analysis of results. PCR offers high sensitivity and specificity, enabling the detection of even minute quantities of viral nucleic acids in clinical samples.

High Sensitivity

Detects low viral loads.

High Specificity

Reduces false positives.

Sequence Analysis

Enhanced with databases.



Polymerase Chain Reaction (PCR)

PCR is one of the greatest advances in molecular biology. It amplifies a single copy of a gene sequence *_ in vitro_*. Amplification is at least a million-fold within a few hours. Viral DNA from a small number of virions or infected cells can be amplified and identified. PCR can also detect viral RNA through reverse transcriptase conversion to DNA. Its **speed**, **sensitivity**, and **versatility** make it a routine diagnostic tool.

- RNA Detection
PCR also detects viral RNA by converting it to DNA using reverse transcriptase.
- Speed
- Sensitivity
- Versatility

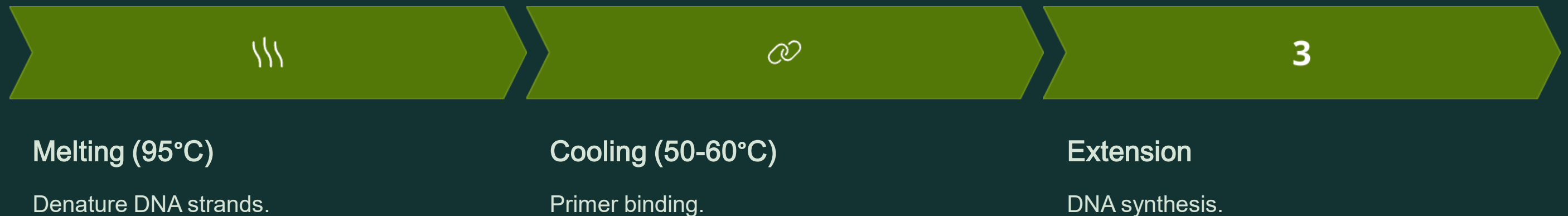
2. Denaturation → Attaches to the single-strand DNA →

2. Primers attach to the two single pieces



The Three Main Steps of PCR

The PCR process requires carefully controlled conditions, including temperature, primer concentration, and nucleotide concentration. There are three main steps: **Melting** the target DNA at 95°C, **Cooling** to 50–60°C for primer binding, and **Extension** of DNA strands from the primers. This generates complementary copies, catalyzed by DNA polymerase. Each cycle doubles the amount of target DNA, leading to exponential amplification.



Viral Cultures: Specialized Applications

Techniques such as animal inoculation (e.g., suckling mice) and egg inoculation are now specialized procedures. They are typically employed when a laboratory has a specific need, such as growing influenza virus in embryonated hens' eggs for vaccine production. These methods require expertise and specialized facilities, and are reserved for applications where other methods are unsuitable.

Animal Inoculation

Used in specific cases.

- Suckling mice

Egg Inoculation

Influenza virus growth.

- Vaccine production

Viral Plaque Assay: Quantifying Infectious Virus

The viral plaque assay is a widely used method in virology for purifying virus populations. A viral plaque results from the infection of a cell by a single virion on a host cell monolayer. This assay requires a susceptible host cell monolayer, where each plaque represents a localized area of cell lysis resulting from viral infection. The number of plaques can be counted to determine the viral titer.

Cell Monolayer

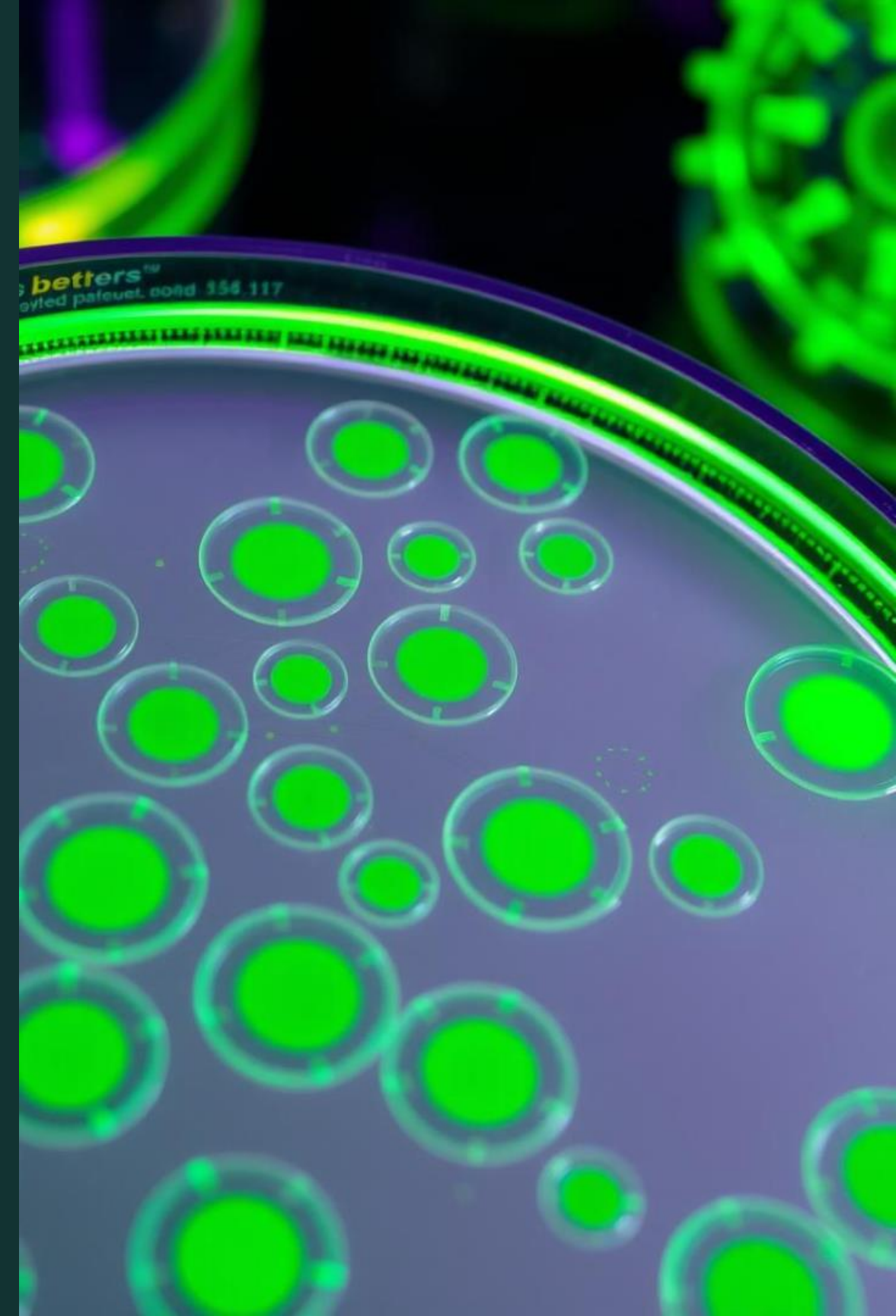
Prepare susceptible cells.

Infection

Infect cells with virus.

Count Plaques

Determine viral titer.



Measurement of Serum Antibodies

Serological techniques identify unknown viruses or viral antigens using antibodies of known specificity. Techniques like ELISA, latex particle agglutination, hemagglutination, and immunodiffusion are employed using known antigens to identify unknown antibodies. Serological tests diagnose acute or chronic infections and provide indications of both clinical and subclinical infections, giving a truer record of total infections.



Diagnosis

Identify infections.



Record

Clinical and subclinical.



Specificity

Using known antigens.



Summery

In summary, effective viral detection relies on a combination of techniques, each with its strengths and limitations. While rapid immunoassays offer simplicity, molecular methods provide enhanced sensitivity and specificity. Proper technique selection, quality control, and data interpretation are crucial for accurate diagnosis and management of viral infections. Future research and development will continue to refine and expand our diagnostic capabilities.

