Chapter 5 - Lesson 4

Virology Techniques

Introduction

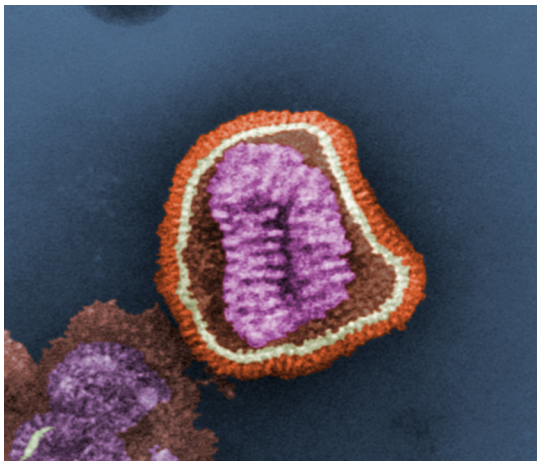
Virology is a field within microbiology that encompasses the study of viruses and the diseases they cause. In the laboratory, viruses have served as useful tools to better understand cellular mechanisms. The purpose of this lesson is to provide a general overview of laboratory techniques used in the identification and study of viruses.

A Brief History

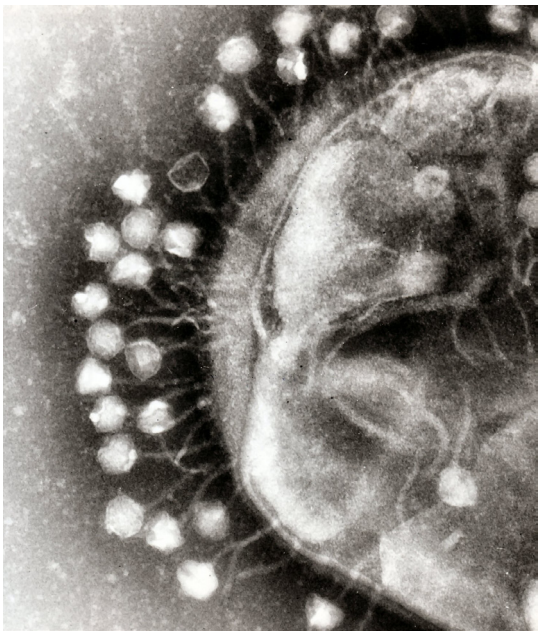
In the late 19th century the independent work of Dimitri Ivanofsky and Martinus Beijerinck marked the beginning of the field of virology. They showed that the agent responsible for causing a serious disease in tobacco plants, tobacco mosaic virus, was able to pass through filters known to retain bacteria and the filtrate was able to cause disease in new plants. In 1898, Friedrich Loeffler and Paul Frosch applied the filtration criteria to a disease in cattle known as foot and mouth disease. The filtration criteria remained the standard method used to classify an agent as a virus for nearly 40 years until chemical and physical studies revealed the structural basis of viruses. These attributes have become the basis of many techniques used in the field today.

Background

All organisms are affected by viruses because viruses are capable of infecting and causing disease in all living species. Viruses affect plants, humans, and animals as well as bacteria. A virus that infects bacteria is known as a bacteriophage and is considered the



This electron micrograph depicts an influenza virus particle or virion. CDC.



Bacteriophage. CDC.

most abundant biological entity on the planet. Many animal disease systems serve well as models for human disease. Many of the techniques used in the study of viruses are the same whether they infect plants or warm- or cold-blooded creatures because the techniques are based more on the virus studied rather than the species affected.

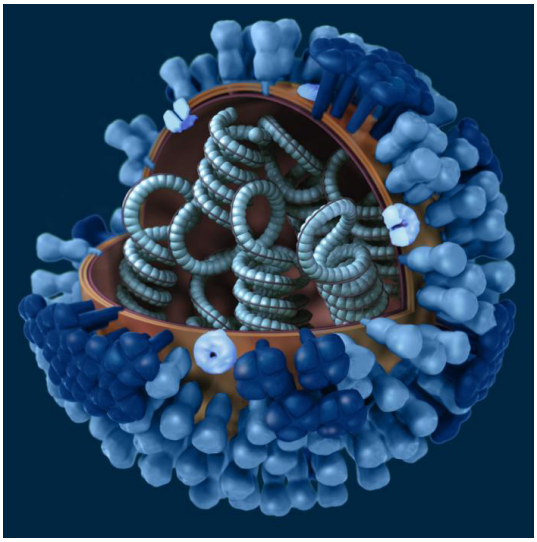
Viruses are considered obligate intracellular parasites since they require living host cells to replicate. Viruses take over or hijack the cellular synthesis machinery in order to reproduce. Viruses are submicroscopic and contain either DNA or RNA as their genome and is enclosed in a protein shell called the capsid. Coded in the DNA or RNA genome of the virus is all the information needed for replication. Some viruses also contain lipids, carbohydrates, and special enzymes that assist in their transmission and replication. Some viruses are enveloped; this envelope is acquired from the host cell either from the cytoplasmic or nuclear membrane. This outer coat is immunogenic, (what the immune system recognizes and what antibody producing cells respond to), and is necessary for the virus to invade a cell.

Laboratory Techniques

Several different methods are used to study viruses and viral diseases, as the field is constantly changing with the discovery of new methodologies and technologies. This section will provide a cursory overview of the most commonly used techniques in diagnostic virology and will conclude with a brief glimpse of virology in research.

Diagnostic virology is concerned with identifying the virus associated with clinical signs and symptoms. Procedures most commonly used include:

1. Detection of a meaningful immune response to the virus (antibody or cell-mediated) by immunologic assay(s)
2. Identification of the agent by staining of specimens or sections of tissue (light and electron microscopy)
3. Isolation and identification of the agent (cell culture or fertile eggs)
4. Detection of viral nucleic acid (probes or amplification).



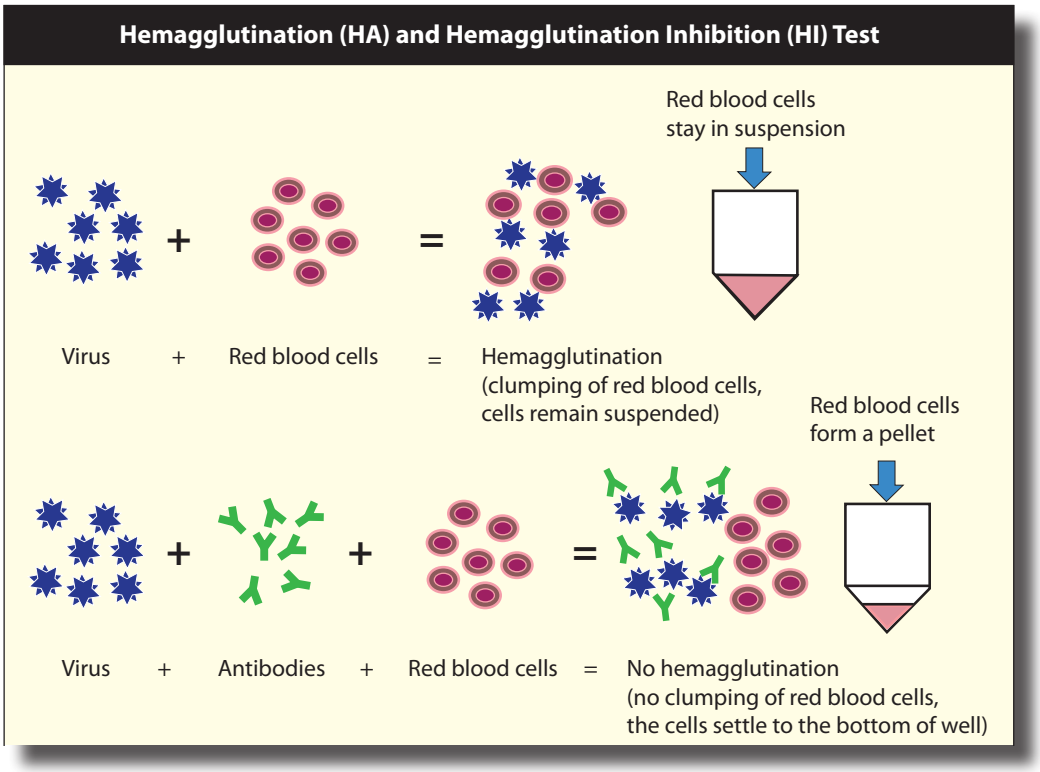
This illustration provides a 3D graphical representation of a generic influenza viron's substructure. A portion of the viron's outer protein coat has been cut away. Dan Higgins, CDC.

Detection of Immune Response

Often, it is difficult to identify a virus in relation to the disease observed, or when conducting a retrospective study of a population to determine exposure to a virus, or when measuring the response of an individual to a vaccine. In these cases, indirect methods of measure are needed, such as measuring antibody response to the virus of interest. Several methods exist for this purpose. A few of the most commonly used methods include:

- Virus neutralization (VN)
- Hemagglutination inhibition (HI)
- Enzyme linked immunosorbent assay (ELISA)
- Indirect fluorescent antibody (IFA)
- Complement fixation (CF)
- Agar-gel immunodiffusion (AGID)
- Agar-gel precipitin (AGP)
- Latex agglutination (LA).

The principles of these assays are fundamentally the same, they depend upon antibody-antigen interactions and consist of a known virus or viral protein, a patient sample (usually serum), and an indicator. If antibodies are present in the patient's serum, they will bind to the virus. If no antibodies are present, no binding will occur. The indicator is observed to determine whether the sample is positive or negative for antibodies.

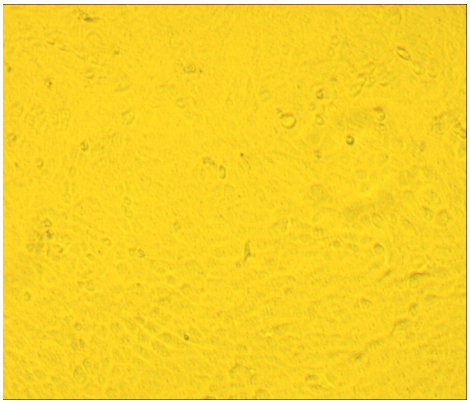


Virus Neutralization

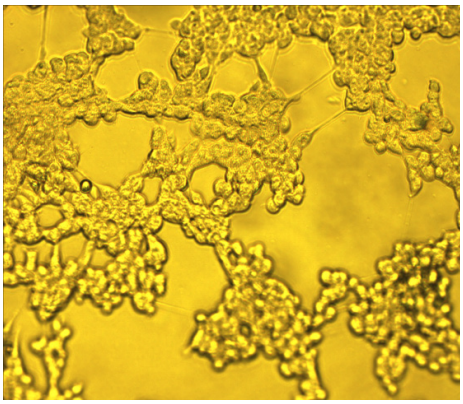
In the virus neutralization (VN) test, the sample of interest is incubated with the target virus and changes in cell culture are observed (called cytopathic effect, CPE). If the sample contains antibodies, it will prevent the virus from growing in the cell culture and no CPE will be observed. If no antibodies are present in the sample, the virus will grow and CPE will be observed.

Hemagglutination Inhibition

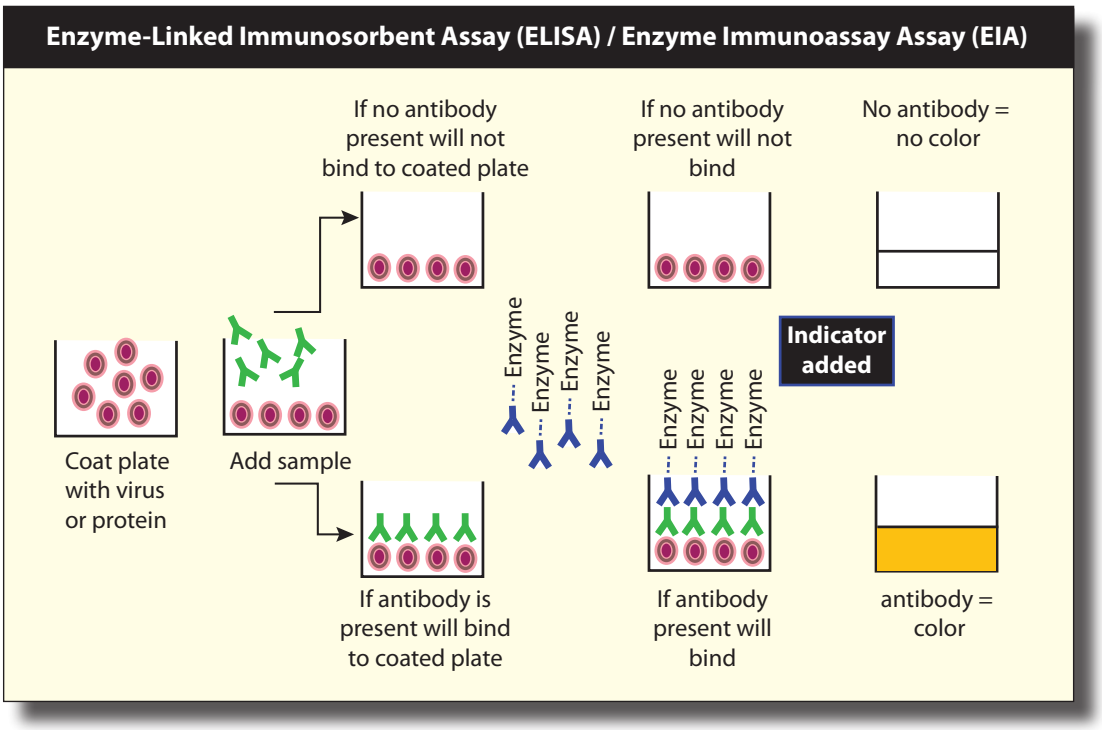
Certain viruses have a protein on their surface that interacts with red blood cells and is able to attach to them. This property is called hemagglutination and the surface protein of the virus is hemagglutinin. The inhibition or blocking of this activity is the basis of the hemagglutination inhibition (HI) test. The most well known virus with this property is the influenza virus. Like the virus neutralization (VN) test, the patient's serum sample is incubated with the virus of interest but instead of growing the virus in cells, red blood cells are added to the virus-serum mix. If antibodies are present, the hemagglutination activity will be blocked; if no antibodies are present the virus will agglutinate (bind together). In this case the red blood cells are the indicator.



Normal bovine kidney (BK) cells



Bovine kidney (BK) cells showing cytopathic effects (CPE) of bovine herpesvirus.

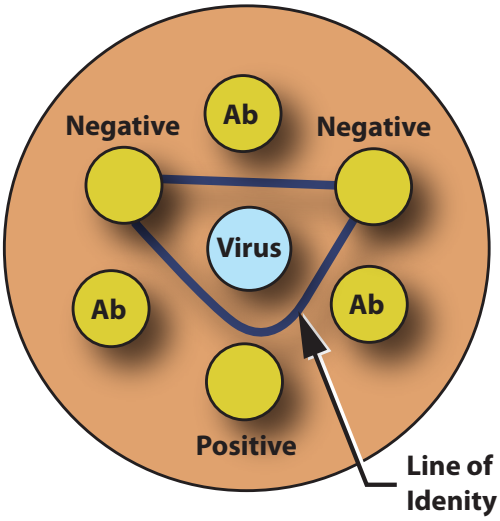


Enzyme-Linked Immunosorbent Assay

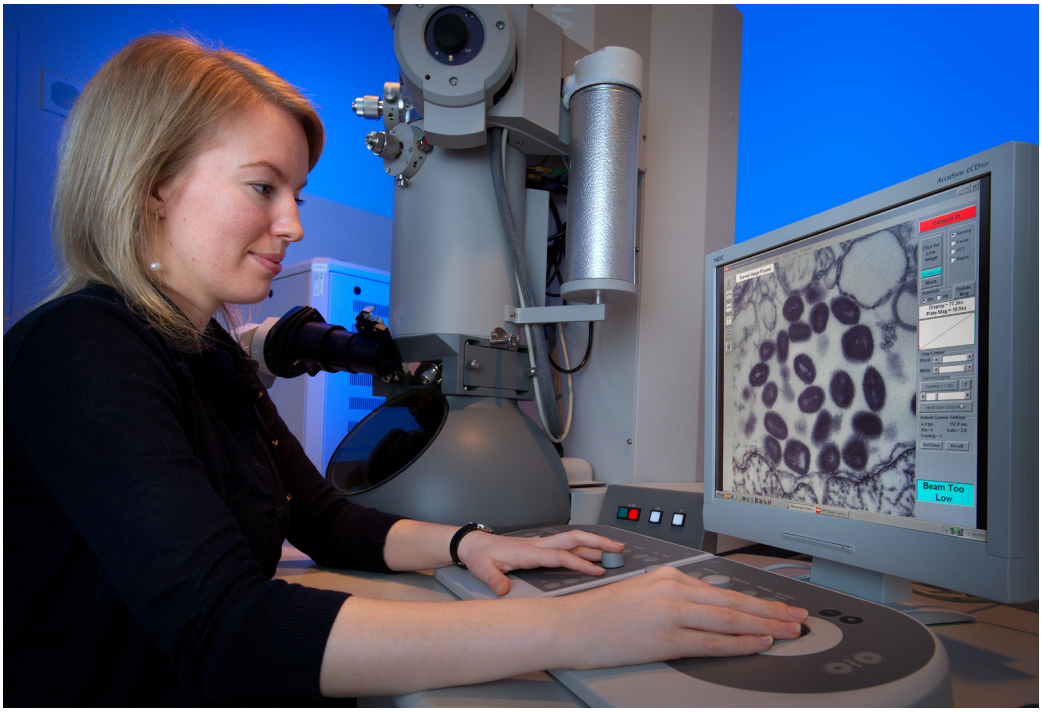
The enzyme-linked immunosorbent assay ELISA is a very popular technique due to the ease of use and low cost. The ELISA consists of plastic wells coated with either the antigen (virus) of interest or a protein specific to the antigen (virus) of interest. The unknown sample (serum) is allowed to bind to the coated well, an antibody labeled with an enzyme is applied, an indicator is added, and then a color change is observed. The presence of color indicates the presence of antibodies and the absence of color indicates the absence of antibodies.

Agar-Gel Immunodiffusions (AGID)

The agar-gel immunodiffusion (AGID), also referred to as an agar gel precipitin (AGP) test, involves the diffusion of virus and antibody through an agar (gelatin-like substance), which will form a line of identity where the antigen-antibody complexes form.



Schematic of an agar gel immunodiffusion (AGID) or agar gel precipitin (AGP) test. "Ab" represents a know antibody to the known virus in the middle



The electron microscope is being used to examine a thin section of the variola virus, revealing some of the structural features displayed by this pathogenic organism. CDC.

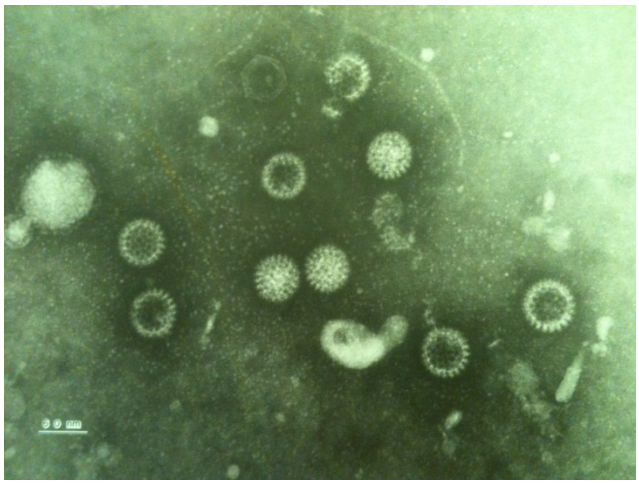
Light and Electron Microscopy

Light Microscopy

Viruses, unlike bacteria, are too small to be seen using a standard light microscope. Therefore, antibodies labeled with an indicator, most frequently peroxidase or fluorescence, designed to identify the virus of interest are used. This label then enables the visualization of the virus cluster (because a single virus is too small to see with a light microscope) with the light microscope, in the case of peroxidase, or an ultraviolet (UV) light microscope in the case of fluorescence.

Electron Microscopy

Another way to identify a virus is with the use of the electron microscope. Since viruses are much smaller than bacteria, a regular light microscope does not provide sufficient magnification to see them. The magnification of an electron microscope (50,000x magnified) provides the ability to see the viral particles. The problem with this method is the lack of sensitivity: a concentration of approximately 10^6 (1,000,000) virus particles per milliliter of fluid is required in order to see the virus of interest. However techniques can be used to improve this, such as immune electron microscopy. With this technique the sample is incubated with an antibody against the virus of interest and the antibody-antigen reaction results in clumps of the virus, which allows for easier visualization. With the advancements in molecular methodologies, electron microscopy is becoming less widely used.



Rotavirus particles as seen under an electron microscope.

Microbiologist is inoculating 10-day of emryonated chicken eggs. CDC.

Virus Isolation

The first step in identification of a viral infection often involves the ability to isolate the virus. The two most commonly used methods are cell culture and fertile chicken eggs. Several problems exist with this technology. One is that the success of isolation is dependent on a viable virus particle. Often, when trying to identify a virus as the source of a disease, the virus is no longer in the process of reproducing itself and is not producing infectious offspring. This method is also highly dependent on optimal collection and sample handling, as many viruses are not very stable outside of the host system. Another problem is the availability of suitable systems, such as the right cell line to be able to grow the suspected virus. Not all viruses grow in the same system and not all viruses grow in cell culture, thus the need for a variety of options.

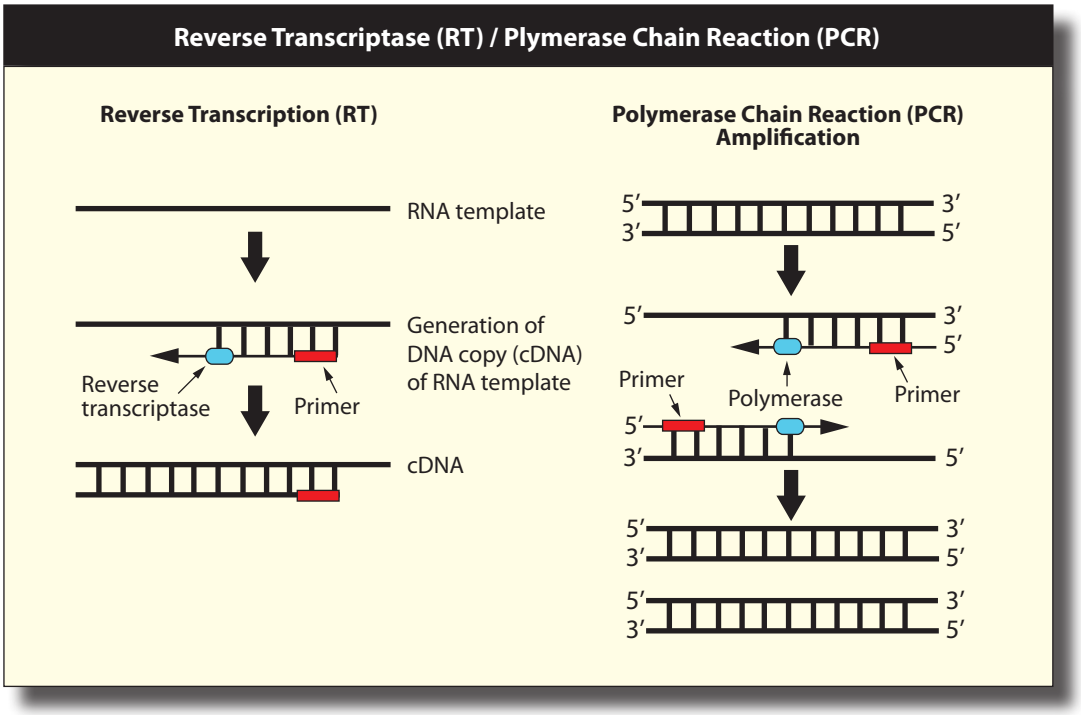
The benefit of virus isolation as opposed to molecular techniques is the possibility of identifying something new in a species. Most molecular methods used today rely on the development of an assay to a known target (virus) and are specific for the particular target of interest. Isolation methods are less specific and whatever can grow will.

Many viruses, when they grow in cell culture systems, will show characteristic changes in the cells from the control (normal) cells. Virus isolation is often a very slow and labor intensive process, thus alternative tests are constantly being developed. The most popular tests at this time are molecular methods, such as polymerase chain reaction (PCR) and real-time polymerase chain reaction. With the increased availability of molecular tests, fewer diagnostic laboratories attempt virus isolations.

Molecular Methods

The area undergoing the most growth is in molecular methods and techniques, particularly in relation to virology. Molecular methods do not rely on the presence of a live virus like virus isolation procedures. These methods detect a piece of the viral genome, making them more sensitive for the detection of viruses.

Molecular techniques are similar across disciplines thus techniques used in the study of viruses are similar to those used in other areas of microbiology such as bacteriology (bacteria) and mycology (fungus).



Traditional molecular techniques are dot-blot, Southern blot, and *in situ* hybridization. These methods are dependent on the use of specific DNA or RNA probes. They are similar in sensitivity to some of the classical methods but are more tedious and expensive, and are not routinely used in diagnostic laboratories.

The technique that has probably had the greatest impact on the field of virology is the polymerase chain reaction (PCR, identification of DNA) and the reverse transcriptase polymerase chain reaction (RT-PCR, identification of RNA) along with the development of better, more standardized methods of nucleic acid purification. The rapid advances and decrease in expense associated with nucleotide sequencing, commercial synthesis of oligonucleotides (primers), and the availability of genetic sequences in public databases have contributed to the increased appeal of these methods. The PCR involves the use of two primers designed to identify a target. In the presence of a DNA polymerase and other components required for DNA amplification, the target sequence of interest, if present, is amplified. The PCR requires double stranded DNA, thus it is useful for identifying DNA viruses. The discovery of reverse transcriptase, an enzyme capable of making a DNA copy of RNA, enabled the ability to expand PCR to include a reverse transcription step (RT) which generates a double stranded target (RNA-DNA), which is then used in the PCR to identify RNA viruses.

Summary

Virological techniques expand beyond diagnostics into the research laboratory. Many animal disease systems are used as models for human diseases. Using several different types of molecular methods, such as cloning and inserting and deleting genetic information, viruses are being engineered in a variety of ways to improve human and animal health. Some of the results of these manipulations are improved vaccines, viruses engineered to carry genetic information for use in gene therapy, as well as for use in cancer treatment. Virology is a constantly growing and dynamic field with much left to discover.

References

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Questions

1. Name three assays designed to identify the presence of antibodies in a patient's serum.
2. Name three assays designed to identify the presence of a virus.
3. What is a limitation with virus isolation?
4. What is an advantage for virus isolation?
5. What is a benefit of molecular assays?