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To control the viral transmission, the identification of the culprit is far more important than anything else. Historically, immunological methods have been used extensively for virus diagnosis until recently. As molecular methods became implemented in viral diagnosis since the 1990s, polymerase chain reaction (PCR)-based molecular methods have become a standard in clinical laboratories, because it is sensitive and readily applicable to diverse pathogens. In addition to molecular and immunological tools, some classical methods such as plaque assay are still used for some viruses. Here, the methods used for viral diagnosis as well as the experimental tools used for virus research will be described. Electron microscopy, which is also utilized for viral diagnosis as well as basic research is described in chapter “Virus Structure.”

4.1 VIRUS DIAGNOSIS

For the diagnosis of virus infection, diverse experimental tools are utilized to detect, measure, and quantify the viral products (ie, protein and nucleic acids). These experimental approaches can be largely divided into two groups: immunological methods to detect viral proteins (antigens) and molecular methods to detect viral nucleic acids. In addition, hemagglutination is often used for some enveloped viruses, such as influenza viruses.

4.1.1 Protein Detection

Proteins are one of the principal components of virus particles. Historically, the viral proteins have been utilized as viral markers, since they are readily detectable by antibodies. Three immunological methods used for the detection of viral proteins (ie, antigens) will be covered here: ELISA, immunofluorescence assay (IFA), and immunoblotting (IB). All three methods rely on the specificity of antigen—antibody binding. Either polyclonal or monoclonal antibodies (Mab) can be used; however, the use of Mab is becoming a standard, as a large number of Mab with their defined epitopes become commercially available.

1. Monoclonal antibodies (Mab) Monospecific antibodies that are made by identical immune cells that are all clones of a unique parent cell. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope.
4.1.1.1 ELISA

ELISA\(^2\) is the most frequently used diagnostic tool for virus detection that combines the exquisite specificity of antigen-antibody binding and the sensitivity of enzyme reaction. In principle, ELISA is a format of analytic biochemistry assay that uses a solid-phase enzyme immunoassay to detect the presence of an antigen in a liquid sample. Commercial ELISA kits are currently used for the diagnosis of human pathogenic viruses including human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infection in clinical laboratories. It can be formatted to detect antigen as well as antibody. Three formats are typically used: (1) direct ELISA, (2) indirect ELISA, and (3) sandwich ELISA.

Direct ELISA: Direct ELISA is used for the quantitation of antigens present in specimens (Fig. 4.1A).

\(^{2}\) ELISA (enzyme-linked immunosorbent assay).
Typically, a 96-well microtiter plate is used as a solid support (Fig. 4.1B). Antigens from the specimens, which are being measured, are attached to the surface of the plate. Then, the primary antibody (ie, antigen-specific) that is conjugated to an enzyme [ie, alkaline phosphatase (AP) or horseradish peroxidase (HRP)] is applied to the well so that it can bind to the antigen. The enzyme linked to the primary antibody converts a chromogenic substrate to a colored product (yellow), when a chromogenic substrate is added. In this format, the antigen attached to the plate is diluted to be limiting, the extent of enzyme reaction (ie, color change) is related to the amount of antigen attached to the plate. As a result, the amount of antigen present in the specimens can be quantitatively estimated. One flaw of the direct ELISA is that the primary antibody conjugates need to be prepared individually for each antigen. Indirect ELISA is designed to obviate this flaw by employing so called secondary antibody conjugate.

**Indirect ELISA**: Indirect ELISA is fundamentally similar to the direct ELISA, except that one additional antibody (ie, secondary antibody) is employed (see Fig. 4.1A). The secondary antibody is specific to the Fc fragment of IgG molecule (see Fig. 5.12) of the primary antibody so that the secondary antibody conjugate can be broadly used. For instance, the secondary antibody specific for the Fc fragment of rabbit primary antibody can be broadly used for any rabbit antibodies, regardless of their antigen specificity. This procedure is dubbed “indirect ELISA,” as antigens are indirectly measured by the use of secondary antibodies. Indirect ELISA can be used for the quantitation of antibodies as well as antigens present in specimens. For the quantitation of antibodies, the primary antibody from specimens is diluted to be limiting so that the extent of enzyme reaction (ie, color change) is related to the amount of antibodies.

**Sandwich ELISA**: Sandwich ELISA or capture assay is used for the quantitation of antigens. Unlike indirect ELISA, two specific antibodies (A and B), instead of one, are required (see Fig. 4.1A). To begin with, a specific antibody A is attached to a 96-well microtiter plate. Then, antigens in the sample are applied to the plate so that antigens could bind to the antibody A attached on a solid support. Then, a specific antibody B is applied so that it could bind to antigens captured on a solid support. Next, the enzyme-linked secondary antibody is applied, as above. Finally, a chromogenic substrate is added for measurement. As the extent of enzyme reaction (ie, color change) is also correlated to the amount of antigen bound to the antibody A, the amount of antigen present in the sample can be quantitatively estimated. This procedure is dubbed “sandwich ELISA,” as the antigen is sandwiched between two antibodies. It is important that two antigen-specific antibodies should bind to two distinct sites on the antigen. Two Mab having distinct binding specificities (ie, epitope) on a given antigen are conventionally used (see Fig. 5.13).

Overall, two formats are practically used: (1) indirect ELISA for the detection of antibodies and (2) sandwich ELISA for the detection of antigens (Fig. 4.1).

### 1.1.1.1 Immunofluorescence Assay

IFA is a microscopic method that can detect and visualize the viral proteins expressed in cells via antigen—antibody reaction (Fig. 4.2). First of all, the virus infected cells are grown on cover glass. To visualize the antigen, the cells are then fixed by paraformaldehyde so that the proteins in the cells are poised to antibody binding. Cells are then permeabilized by a proper detergent such as triton X-100. A specific antibody (eg, patient sera) is applied on the surface so that the viral antigen is recognized by the antibody. Then, the secondary antibody, which is specific to Fc fragment of IgG molecule of the primary antibody, is applied. Since a fluorescence dye is linked to the secondary antibody, the antigen can be visualized under a fluorescence microscope. Subcellular localization of the viral antigen can be revealed by IFA. Moreover, two or even three antigens can be readily visualized simultaneously by using two to three distinct antibodies. Currently, IFA is used for research rather than diagnostic purposes. Nonetheless, in the early days when ELISA kit was not yet commercially available, IFA was used for diagnostic purposes by using patient sera as primary antibody.

### 1.1.1.2 Immunoblotting

IB is a convenient tool extensively used in laboratories. IB, also called Western blot, can readily detect proteins transferred onto a solid support (ie, nitrocellulose membrane). It combines the protein resolving capability of SDS gel electrophoresis with the specificity of antigen—antibody binding (Fig. 4.3). First, the proteins in mixtures (eg, cell lysates) are resolved with SDS—PAGE. Next, the proteins on the gel are transferred to a membrane by electric current; they are literally “blotted” to the membrane, as the name implies. Subsequently, the proteins on the membrane are detected by a series of antibody reactions: first with primary antibody for the specific detection of antigens, then with the secondary

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3. **SDS—PAGE** Polyacrylamide gel electrophoresis (PAGE), is a technique widely used to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. SDS—PAGE is a method to separate proteins following denaturation by SDS, a detergent.
antibody, which is linked to an enzyme (AP or HRP) that allows visualization. Finally, the protein bands on the membrane are visualized by the chemiluminescence method, which is better known as ECL.

### 4.1.2 Nucleic Acids Detection

Besides proteins, the nucleic acid component of the virus particles could be readily detectable for the diagnostic purpose. Two methods are frequently used for the detection of the nucleic acids: PCR and real-time PCR (RT-PCR).

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4. **ECL (enhanced chemiluminescence)** ECL is a common technique for a variety of detection assays in biology. An antibody that is conjugated to an enzyme (either HRP or AP) is used. The enzyme catalyzes the conversion of the enhanced chemiluminescent substrate into a sensitized reagent, which emits light. ECL allows detection of minute quantities of a biomolecule.
4.1.2.1 PCR

PCR has dramatically revolutionized viral diagnosis. Considering its versatility, PCR is a truly handy technology, because it requires only one instrument (ie, thermocycler) and only few reagents (Fig. 4.4). More importantly, PCR allows the diagnosis of the viral infection at very low titer (eg, \(10^6\) particle per mL). For instance, clinical diagnosis of early phase of HIV infection, when neither the viral antigens nor antibodies are detectable, can be accomplished by PCR.

The method relies on thermal cycling, consisting of cycles of heating and cooling of the reaction for DNA melting and enzymatic amplification of the DNA (Fig. 4.5). As PCR progresses, the DNA serves as a template for amplification, setting in motion a chain reaction in which the DNA is exponentially amplified. Primers containing sequences complementary to the target region enable specific and repeated amplification. A heat-stable Taq polymerase is another key component for the repeated amplification. One shortcoming is that the result of PCR is only semiquantitative, as it inherently involves amplification. To overcome this shortcoming, RT-PCR was developed.
4.1.2.2 Real-Time PCR

RT-PCR is an innovative advance of PCR technology. As the name implies, RT-PCR is built with a technology that is capable of monitoring PCR product as it is being amplified. Probing technology is the critical feature of RT-PCR. A few kinds of probing technology have been commercialized: TaqMan probe and Molecular Beacon probe. Here, for brevity, the TaqMan technology will be described (Fig. 4.6A). It represents a specially designed oligonucleotide probe that has a fluorophore attached to one end, while a quencher is attached at the other end. When it is bound to the template during the annealing step, no fluorescence is emitted, since fluorescence is quenched. However, during the polymerization step, the fluorophore is cleaved by 5’ to 3’ exonuclease activity associated with Taq polymerase, then the fluorescence can be detected. As the amplification proceeds, fluorescence emitted correspondingly increases. The real-time monitoring capability has transformed the PCR from qualitative to quantitative. For instance, the use of a standard DNA with known copy number in parallel allowed quantitation within a less than twofold error range (Fig. 4.6B).

4.1.3 Hemagglutination

Hemagglutination is used for the diagnosis of some enveloped viruses such as influenza viruses. This method relies on the specific feature of some enveloped viruses that can adsorb to red blood cells (RBCs). Specifically, hemagglutinin (HA), an envelope glycoprotein of some enveloped viruses, imparts this property. In the absence of virus particles, RBCs precipitate by gravity to the bottom of the well, giving rise to a distinct red-colored dot in a conical shaped well (Fig. 4.7A). In the presence of virus particles, RBCs clump together as a result of interaction between HA proteins of virus particles and RBC, leading to a lattice formation. In this case, as RBCs are dispersed as a clump, a red dot is not formed. In a given sample, a red dot will appear beyond a certain dilution fold. To carry out a hemagglutination assay,

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5. Hemagglutinin (HA) Hemagglutinin refers to a substance that causes RBCs to agglutinate or to clump together. This process is called hemagglutination.
a twofold serial dilution of virus-containing samples is dispensed into individual wells of a 96-well microtiter plate (Fig. 4.7B). Then, aliquots of RBC are added to each well. The highest dilution at which clumping is observed is regarded as the HA titer of the sample. The virus titer in a sample can be estimated by multiplying the dilution fold. In a standard condition, 1 HA unit corresponds to $10^4$ particles per mL.

Hemagglutination is a classical method for viral diagnosis, but is still used for diagnosis of influenza virus today. One outstanding advantage of this method is that it does not require any equipment. Moreover, it is a robust and rapid diagnostic tool, but the sensitivity is somewhat limited.

Now, we will discuss experimental methods used in virus laboratories for research purposes, including virus cultivation, quantitation, purification, and genetic analysis.

### 4.2 CULTIVATION OF VIRUSES

To investigate viruses and their life cycles, one should be able to propagate the virus in a laboratory. Animal viruses, with a few exceptions, are cultivable in the laboratory by using tissue culture. A variety of animal and human cell lines are now available and used for virus cultivation. In addition to tissue culture, embryonated eggs and experimental animals are used for virus cultivation in certain circumstance.

#### 4.2.1 Tissue Culture

Animal viruses are typically grown by using tissue culture in laboratories (Fig. 4.8). In most cases, cell lines, instead of tissue, are used. Cell lines refer to immortalized cells that have acquired the ability to proliferate indefinitely (Box 4.1).
FIGURE 4.8  Primary cells versus immortalized cell lines. (A) Preparation of primary cells. A tissue (eg, embryo) is isolated from a subject by surgery, minced by a knife, and treated with collagenase. Cells are filtered and centrifuged. The cells collected by centrifuge are transferred to a plate, and cultivated by adding culture medium with 10% fetal bovine serum (FBS). Cells are regularly transferred to a new plate by splitting cells after treatment with trypsin. (B) Immortalized cell lines. Cells are typically grown as a monolayer in a tissue culture flask with a cell culture medium [eg, Dulbecco’s modified eagle medium (DMEM) with 10% FBS]. Most animal cells divide approximately once a day. When the monolayer becomes confluent, cells are treated with trypsin to detach from the plate, and then transferred to multiple new plates. The transferred cells become attached to a new plate within a day and resume their growth in a new plate.

BOX 4.1 Primary Cells and Cell Lines

Cell lines are the most convenient methods for virus cultivation in a laboratory. However, in a case where cell lines susceptible to the virus infection are unavailable, primary cells explanted directly from a living animal or human are the only choice for virus cultivation. What are the characteristic differences between primary cells and cell lines?

A diagram depicting steps involved in preparation of primary cell versus immortalized cell line. Cell strains are cells that have a limited proliferation capability and can be immortalized. Cell line can be directly prepared from cancerous cells that are already immortalized during cancer development.
In fact, cell lines from diverse tissue origins (human cancer) were established. For instance, HeLa cells are derived from tissue of human cervical carcinoma (see Box 7.4). For most human viruses, cell lines that support the viral replication are available, with a few exceptions. In the latter case, primary cells are instead used, albeit it is cumbersome to prepare the primary cells taken directly from a living animal.

### 4.2.2 Embryonated Eggs

Before tissue culture technology were established in the 1950s, embryonated eggs had been used for cultivation of some viruses such as influenza virus during the 1930s—1950s. Even today, embryonated eggs are utilized for the propagation of influenza virus; in particular, for vaccine production (see Fig. 25.10). A chick embryo at 10—14 days after fertilization constitutes diversely differentiated tissues including amnion, allantois, chorion, and yolk sac (Fig. 4.9). Viral inoculum can be administered into the allantoic cavity of embryonated eggs, and a few days later, the progeny viruses can be harvested from the amniotic fluids. Although embryonated eggs have been largely replaced by tissue culture these days, it is still used in a laboratory to obtain high viral titer for some viruses such as influenza virus and Sendai virus.

#### FIGURE 4.9 Embryonated eggs for virus cultivation.

(A) A diagram showing the anatomic details of embryonated eggs. Diversely differentiated tissues at day 10–14 after fertilization are denoted. (B) A virus inoculum is being injected to an embryonated egg.

### 4.2.3 Experimental Animals

According to Animal Welfare Acts, animal usage for any experiments should be minimized. Nonetheless, animals are often used in virus research laboratories under a certain circumstance. For a given virus, both natural and nonnatural hosts are used as experimental animals. For instance, mice are often used as an experimental animal for the infection of influenza virus, although mice are not the natural host for influenza virus. Moreover, experimental animals are indispensible for antiviral drug development and vaccine development (see chapters: Vaccines and Antiviral Therapy). For instance, cell culture experiment is inappropriate for studying infection pathology, since only one type of cells is cultured. By contrast, in animals, all kinds of cell types including immune cells are present in physiological circumstance.
Consequently, when the discrepancies of results between the studies of cell culture and those of experimental animal are noted, the results from animal studies are respected.

4.2.4 Animal Models

Humans are the only natural host for some human pathogenic viruses such as HIV. Since human beings cannot be the subject of experimentation, it is crucial to find alternatives that can reflect features manifested in human infection. Such alternatives are collectively called animal models. Although animal models are not the natural host of human viruses, they can be experimentally infected by human viruses, and are amenable to experimental analysis. In practice, mice are the foremost choice, as they are small, affordable, and genetically well characterized. Although mice are not the natural host of influenza virus, mice can be experimentally infected, for instance, by the human influenza virus. On the other hand, for some viruses, such as HIV, primates (ie, chimpanzee) are the only choice, as they are the only animal permissive of HIV infection.

4.3 QUANTIFICATION OF VIRUSES

The quantification of viruses involves counting the number of virus particles in a sample. It is an essential method in virus research laboratories. In principle, two methods are used for the quantitation of virus particles or virus infectivity: (1) molecular methods such as PCR, and RT-PCR and (2) virological methods such as plaque assay, end-point dilution assay, and hemagglutination assay. Although the molecular methods have largely replaced the classical virological methods, they cannot measure the infectivity, a defining feature of the virus particle.

4.3.1 Plaque Assay

Plaque assays are the standard method that have long been used to determine the virus titer (ie, infectious dose) (Box 4.2). It determines the number of plaque forming units (pfu) in a sample. Typically, 10-fold serial dilutions of the...
virus stock are inoculated into each plate (Fig. 4.10). The virus infected cell will lyse and spread the infection to adjacent cells. As the infection-to-lysis cycle is repeated, the infected cell area will form a plaque which can be seen visually. The pfu/mL result represents the number of infectious particles in the sample, assuming that each plaque is caused by a single infectious virus particle. Note that plaque assay is restricted to the viruses that induce cell lysis or death, such as picornavirus, influenza virus, and herpesvirus.

In addition to pfu, MOI is frequently used as the unit of virus titer. It refers to the number of virus particles loaded per cell for infection experiment. Low MOI (eg, 0.1) is used for the experiment where only a subset of cells needs to be infected. In contrast, high MOI (eg, $10^3$) is used for the experiment where the vast majority of cells need to be infected.

### 4.3.2 End-Point Dilution Assay

Plaque assay is limited to only a subset of animal viruses that can lead to cell lysis, forming plaques on the monolayer of cells in a cell culture plate. In fact, many animal viruses do not form plaques on the monolayer, but nonetheless induce a discernible CPE. These morphological changes that are observable under microscope can be exploited for quantitation, a method termed “end-point dilution assay.” Likewise, 10-fold dilutions of virus stock are individually added to each well of the cell monolayer (Fig. 4.11). A few days later, wells are examined to see whether CPE is present. Then, the fold of dilutions that led to the CPE in 50% of wells seeded can be estimated. This value of virus titer is termed, $TCID_{50}$. For instance, $TCID_{50} = 100$ means that 100-fold dilution of a given virus stock is estimated to induce CPE in 50% of wells seeded. Note that the specific CPE can be diverse, depending on viruses, ranging from vacuole formation to cell lysis.

### 4.4 PURIFICATION OF VIRUSES

Isolation and purification of virus particles from specimens (ie, tissues or bloods) is essential for the characterization of the virus of interest. For this purpose, virus particles are routinely purified by two methods: either by biological
methods, such as plaque isolation, or by physical methods, such as centrifugation. Importantly, plaque isolation is used for the qualitative purification of (genetically identical) virus particles, while centrifugation is used for the quantitative purification of (biochemical purity) virus particles.

4.4.1 Plaque Isolation

Virus particles can be purely isolated from a single plaque by plaque assay. For instance, a sample can be taken from a single plaque by pipette tip. In fact, the amount of virus particles taken by a tip represents only a trace amount of virus particles. This sample is used as an inoculum to propagate some quantity of virus particles by using cell culture. Plaque-purified virus is considered to be genetically identical. Note that this is only for plaque forming viruses.

4.4.2 Centrifugation

Viruses can be biochemically purified from specimens by physical means such as centrifugation. Centrifugation is an experimental method used to separate objects (ie, virus particles) from specimens by centrifugal force. For instance, a tube containing a sample in a density medium such as sucrose is subjected to centrifugal forces by spinning. Centrifugal force applied to the sample is termed RCF (relative centrifugal force). For instance, 1000 \( \times g \) (gravity) means that 1000-fold of gravity force is applied by centrifugation. The migration speed of objects by centrifugation is determined by size, shape, and density of particles as well as RCF.

A method called “density gradient centrifugation” is used for isolation of virus particles. The density gradient of sucrose is prepared from top to bottom of the centrifuge tube. Commonly, sucrose or cesium chloride is used as a density medium. Then, two methods of separation are used: (1) rate-zonal separation and (2) isopycnic (equilibrium) separation (Fig. 4.12).

Rate-zonal separation. The objects in the density medium migrate depending on its size, even though their density is identical. The bigger the size of the particle, the faster it migrates. Specifically, a sample containing viral specimen is loaded onto the top of the preformed density gradient (eg, 10–50% sucrose), and then subjected to centrifugation for a few hours (Fig. 4.12A).

The virus particles migrate or sediment depending on their size, and form a band along the density medium (Fig. 4.12B). By taking a fraction corresponding to the virus-containing band, the virus particles can be biochemically
purified. The sedimentation behavior of an object is often referred to as the sedimentation coefficient \( S \), which reflects the relative sedimentation properties of a particle (or molecule) in a standard condition. Practically, it can be considered to simply reflect the size of an object. For instance, an eukaryotic ribosome has a value of 80S.

Isopycnic (equilibrium) separation. When subjected to centrifugal force, an object having a certain density migrates until the density of the surrounding medium is equal to its own density (i.e., equilibrium). This method of separation is termed “isopycnic separation.”

What is a practical difference between rate-zonal separation and isopycnic separation? In rate-zonal centrifugation, the density gradient is premade before centrifugation, while in isopycnic separation, the density gradient is made during centrifugation. As a result, the former takes only a few hours (1–4 h), while the latter takes much longer (>16 h). More importantly, the former is used to separate an object by its physical size, while the latter is used to separate an object by its density.

4.5 GENETIC MANIPULATION OF VIRUSES

To gain insights into the molecular mechanisms of virus genome replication, the effect of virus gene mutations on virus replication needs to be examined. Recombinant DNA technology had been implemented in virology in the 1970s, and as a result, “molecular virology” was established as a subdiscipline of virology. To get started, one needs to obtain the full-length DNA of the viral genome (or the cDNA copy of RNA virus genome), and insert the cDNA into an appropriate plasmid vector to express the viral genes. The plasmid, that is capable of inducing the viral genome replication, is

10. Sedimentation coefficient The unit of sedimentation coefficient represents the size of the particle that precipitates upon centrifugation. It is also called Svedberg number (S).
termed a “replicon.” For instance, to study the function of a gene, a specific mutation is introduced in a replicon. Comparison of the phenotypes of the mutant to that of the wild-type will uncover the function of the gene in the virus life cycle (Fig. 4.13).

Molecular virology is a powerful tool for genetic analysis of viral genes. In particular, PCR-mediated mutagenesis enables us to generate all kind of mutations on the replicon plasmid. For instance, one could make a deletion or insertion or substitution mutation in a gene. Molecular virology is also amenable to RNA viruses by inserting cDNA of a full-length RNA genome into appropriate expression plasmid vectors (see Box 11.1). By using a replicon, the mutation analysis of RNA viruses as well as DNA viruses can be carried out.

4.6 LABORATORY SAFETY

Precautions are necessary to prevent laboratory hazards. In particular, workers in clinical laboratories handle human serum and other body fluids that may contain HIV, or HBV or HCV. Proper laboratory practices involve wearing gloves and laboratory coats. On the other hand, research laboratories handling human pathogens, such as AIDS virus and Ebola virus, need to have a biosafety facility. The biosafety facility is essential not only to prevent laboratory infection of investigators but also to preclude spread of laboratory strains to community. A biosafety facility can be classified by the relative danger as biological safety levels (BSL). The levels of containment range from the lowest biosafety level 1 (BSL-1) to the highest level 4 (BSL-4) (Fig. 4.14). Higher numbers indicate a greater risk to the external environment. At the lowest level of biocontainment, the containment zone may only be a chemical fume hood. At the highest level the containment involves isolation of an organism by means of building systems, sealed rooms, sealed containers, positive pressure personnel suits (sometimes referred to as “space suits”) and elaborate procedures for entering the room, and decontamination procedures for leaving the room. In most cases this also includes high levels of security for access to the facility, ensuring that only authorized personnel may be admitted to any area that may have some effect on the quality of the containment zone.

11. Replicon A replicon in genetics is a region of DNA or RNA, that replicates from a single origin of replication. Here, it refers to a plasmid construct that can induced the viral genome replication, when transfected into cells.
The biosafety level for a given pathogen is stipulated by government authorities (e.g., Centers for Disease Control in the United States). For instance, BSL-2 facility is required for studies of pathogens that cause only mild disease to humans or are difficult to contract via aerosol in a laboratory setting, such as HIV, HBV, and influenza virus. BSL-3 facility is required for studies of pathogens that cause severe to fatal disease in humans but for which treatments exist, such as SARS-coronavirus and West Nile virus. On the other hand, BSL-4 facility is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections, agents which cause severe to fatal disease in humans for which vaccines or other treatments are not available, such as Ebola virus, Marburg virus, and various other hemorrhagic diseases.

4.7 PERSPECTIVES

Experimental tools for viral diagnosis have dramatically evolved from morphological and immunological methods to current molecular methods. Nowadays, molecular diagnosis (i.e., PCR-based methods) is in a mainstay of viral diagnosis, owing to its sensitivity, reliability, and lower cost. Nonetheless, classical methods such as ELISA and hemagglutination assay are still used in many clinical settings, because of their convenience. On the other hand, advances in recombinant DNA technology have established “molecular virology,” which studies the virus life cycle and its impact on the host at the molecular level. In fact, the introduction of molecular technology led to the establishment of molecular virology that has prevailed in the field of virology discipline for past decades. As a result, many details of the virus life cycles of human pathogenic viruses are unraveled. Despite these advances, preventive vaccines and effective antiviral treatment for many human viral diseases are not yet available, including vaccines for HIV, HCV, and Ebola virus. To address these unmet medical needs, several gaps in viral methods need to be addressed. In particular, the importance of animal models for human pathogenic viruses should not be underestimated, because animal models are critically important for studies involving vaccine development and antiviral drug discovery as well as for studies of the infection pathology. Lastly, a biosafety facility becomes essential for virus laboratories handling human pathogenic viruses such as HIV and Ebola virus.

4.8 SUMMARY

- **Nucleic acid diagnosis**: Nucleic acid diagnosis such as PCR, and RT-PCR are mainly used for the detection of viral DNA or viral RNA.
- **Immunological methods**: ELISA, IFA, and immunoblotting are used for the detection of viral antigens.
- **Virus cultivation**: Tissue culture by using cell lines is a convenient method for virus cultivation for most animal viruses.
- **Virus quantitation**: Plaque assay and end-point dilution assays are historically used for virus quantitation. Hemagglutination assay is used for some enveloped viruses such as influenza virus. These classical methods are now largely replaced by molecular methods such as PCR.
- **Molecular virology**: A viral replicon, the plasmid construct capable of inducing viral genome replication, needs to be established for molecular studies. Introduction of mutations in a replicon construct enable us to examine the function of a gene of interest.

**STUDY QUESTIONS**

4.1 Indirect ELISA can be used for the quantitation of either antigen or antibody. How differently do the ELISA procedures proceed?

4.2 Immunological methods utilized for viral diagnosis involve a solid support onto which the viral antigens are bound or presented. What kinds of solid supports are utilized for three immunological methods? (1) ELISA, (2) IFA, (3) Immunoblotting.

4.3 Transfection of viral replicons is frequently used for the investigation of certain human viruses. Please state the advantages of using replicons for the examination of virus genome replication.

**BOOK CLUB**

- Boose, J., August, M.J., 2013. To Catch a Virus. ASM Press, Washington, DC, 364 p.
  
  Highlight: A historical account of virus discovery is vividly captured by authors. From the discovery of Yellow fever virus, the first human virus discovered, to the discovery of HIV, this is a wonderful reading for anyone interested in early day of virus discovery.

**INTERNET RESOURCES**

- CDC (Centers for Disease Control) site for biosafety: [http://www.cdc.gov/biosafety/](http://www.cdc.gov/biosafety/)