Immunological Detection and Characterization

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1 Introduction

Immunological methods are valuable strategies for the diagnosis and characterization of viral infections. These methods rely on antigen–antibody interactions, and they can be adapted to allow direct detection of the virus (antigen detection) or to identify the host's immune response to the virus infection (antibody detection). The methods are also used to identify and characterize virus isolates following in vitro or in vivo propagation. Although molecular detection is often more sensitive than antigen detection, immunological methods still have an important role in the study of the epidemiology, pathology, and assessment of clinical disease associated with viral infection.

The development of simple, rapid, and often relatively inexpensive antigen detection test kits has revolutionized both clinical care and laboratory practice. An understanding of various detection methods is increasingly important in the design and interpretation of epidemiologic studies. The vast array of laboratory tests now permits enhanced detection of viral antigens, although the clarification of the classic issue of "causation" of disease remains blurred.

The significance of detection or lack of detection of a virus or viral antigen remains difficult to interpret. Isolation of a virus from a normally sterile site, such as tissue, cerebrospinal fluid (CSF), or blood, is generally highly significant and usually establishes the etiology of the infection. The identification of certain viruses, such as influenza or respiratory syncytial virus (RSV) in respiratory specimens, also is diagnostic because an asymptomatic carrier state has not been shown to exist. However, prolonged and generally asymptomatic excretion or shedding of other viruses can make the determination of the effect of a particular virus on the disease process very difficult. Viruses such as cytomegalovirus (CMV), adenoviruses, and enteroviruses are shed in disease states but also may be shed asymptomatically for prolonged periods of apparent good health. An additional complicating factor is the differentiation of primary infection from reactivation of disease, a problem common to the study of infections with viruses such as herpes simplex (HSV) or CMV, especially in immunocompromised hosts such as transplant recipients or patients infected with HIV. Interpretation of laboratory results in such situations requires a thorough understanding of the pathogenesis and epidemiology of the virus.

Failure to detect a virus or viral antigen does not necessarily mean that the virus was not present previously or did not cause disease. Although failure to detect a virus may be a result of inappropriate or inadequate specimen collection and handling, it may also be a function of the time course of the disease, the age or antibody status of the patient, and the technical resources available to detect or cultivate the virus. The investigator today has many options to diagnose the presence or past presence of a viral infection, but careful epidemiologic and laboratory studies are still required to ultimately link the viral agent to a specific disease process.

2 Historical Background

The recognition of immune-mediated virus neutralization dates back to the late 1800s when Sternberg extended observations of other scientists of the time and described the neutralization of vaccinia infectivity using serum from a recently vaccinated calf [1]. It was another several decades before diagnostic viral serological methods were developed, including complement fixation in the 1930s and hemagglutination inhibition in the 1940s [2]. Immunofluorescent methods for detecting virus antigen were developed in the 1950s by labeling virus-specific antibodies with a fluorescent reporter such as fluorescein isothiocyanate [3]. A little more than one decade after the initial description in 1959 of the

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radioimmunoassay [4] for detecting human plasma insulin, the method was adapted for virus antigen detection [5]. The radioisotope used as a reporter was replaced later during the 1970s with enzymes such as alkaline phosphatase and horseradish peroxidase that allowed colorimetric detection [6]. The method was also readily modified (see below) to allow detection of antibody. A further modification of immunoassay detection methods followed with the use of substrates that allowed detection of the antigen–antibody interaction via measurement of a chemiluminescent reaction.

The diagnostic procedures used for evaluation of specific viral infections are presented in individual chapters of this book. However, certain common aspects of immunological methods used for virus diagnosis are important for the design and implementation of epidemiologic studies. These general issues are addressed below.

3 Principles of Antigen–Antibody Interactions

An antigen is defined as a substance capable of stimulating an immune response when introduced into the body (e.g., of an immunized animal). When the immune response is the production of antibody, the generated antigen-specific antibody can bind directly to the antigen. The portion of the antigen recognized by and specifically interacting with the antibody molecule is called an epitope. An antigen may have more than one epitope. When this occurs the epitopes either may be distinct molecular structures (and thus distinct epitopes) or they may be the same molecular structure (the same epitope) repeated many times. The portion of the antibody interacting with the antigen is called a paratope. The ability of the epitope and paratope to bind to each other is the basis of the specificity of an antigen–antibody interaction.

The binding of antibody to antigen is thought to be the result of electrostatic and van der Waals bonding over short distances, with the kinetics of the reaction following the law of mass action. The law can be represented mathematically as follows:

$$K_d = [Ag \cdot Ag] / [Ag]$$

where K_d is the dissociation constant, [Ag · Ag] is the concentration of the antigen–antibody complex, [Ag] is the antigen concentration, and [Ab] is the antibody concentration.

From this law, the formation of antigen–antibody complexes reaches equilibrium, and the amount of complex formed is proportional to the concentration of the antibody and antigen present. The binding constant is a measure of the strength of interaction between one epitope and one paratope and is also referred to as the affinity of the antibody. The higher the affinity of a paratope for a corresponding epitope, the greater the strength of binding and the lower the K_d value.

A related concept is antibody avidity, which is a measure of the overall binding strength of an antibody to the corresponding antigen. Antibody avidity is correlated to its affinity, but it is also affected by the number (or valency) of interactions between the antibody and antigen. For IgG antibody, the valency is up to two. Since the antigen–antibody interaction is in equilibrium, the presence of multiple binding interactions can maintain the antigen–antibody complex once formed during periods of dissociation between an epitope and paratope.

There are many factors that affect the interaction between an antigen and antibody and the ability to one or the other in a diagnostic assay. These include the temperature of the reaction conditions and the ionic strength of the solutions used in the assay. As noted above, antibody affinity and concentration are particularly important when developing an antigen detection assay. The relative concentration of antigen-specific antibody is lowest in postinfection sera. It can be increased through hyperimmunization with the antigen to generate polyclonal antisera, and the relative concentration can be increased further by affinity purification of the antibody. The highest relative concentrations are reached through the generation of monoclonal antibodies, and selection of high-affinity monoclonal antibodies for use in diagnostic assays is now widely used in the development of antigen detection assays [7].

Another factor that can influence the assay development is nonspecific interactions between the antibody and other substances in the reaction mix (e.g., attachment to the reaction vessel, other microbial antigens). Although these are generally low-affinity interactions, the concentrations of the competing substances can be high enough to affect the readout of the assay. Many of the low-affinity interactions can be removed by washing steps, use of blocking reagents and use of lower concentrations of antibody, but each antibody used in a diagnostic assay should be evaluated for the presence of these nonspecific reactions. In addition, the use of appropriate controls can identify problems with assay performance [8].

4 Specimen Collection

The appropriate collection of specimens is of the utmost importance for the successful identification of viruses in clinical samples. The source of the specimen, the timing of collection in relation to onset of symptoms, the rapidity and method of delivery to the laboratory, and the clinical and epidemiologic data provided to the laboratory all are important variables that relate to the likelihood of successful identification of a viral pathogen. Knowledge of the restrictions of the diagnostic assay to be used is also important; many antigen detection assays are only approved for use when applied to a limited number of clinical sample types. For example, some influenza antigen assays should only be used with nasal swabs while other assays are approved for detection

of influenza in a broad array of respiratory samples types, including nasal aspirate, nasopharyngeal swab, throat swab, and bronchoalveolar lavage [9].

4.1 Source

The clinical syndrome caused by a virus and its pathogenesis of infection determine the specimen(s) that is most appropriate for virus identification. Viruses that primarily cause disease at a mucosal surface or cause vesicular skin lesions generally can be identified in specimens taken from those sites. However, viruses causing generalized or congenital disease or causing symptoms in an internal organ (e.g., central nervous system) often can be identified in specimens taken from multiple different sites. Viruses that cause respiratory tract disease such as influenza viruses, RSV, and rhinoviruses are most frequently identified in samples of respiratory secretions; viruses that cause gastroenteritis, such as rotaviruses, caliciviruses, and astroviruses, are identified in fecal specimens; and viruses that cause generalized or congenital diseases, such as measles, CMV, and mumps, are identified from respiratory secretions, urine, and blood. However, antigen detection methods may not be available for some of these different sample types. Furthermore, some samples may be more likely to yield a positive sample than others (e.g., fecal specimen vs. rectal swab for rotavirus and bronchoalveolar lavage for respiratory viruses in immunocompromised patients) [10, 11]. The reader is referred to specific chapters for information on the ideal specimen type for specific viruses.

4.2 Timing of Collection

Specimens to be used for virus identification should be obtained early in the course of the illness. For many viral infections, viral shedding begins before the onset of symptoms, peaks during the illness, and disappears around the time that symptoms resolve. There are notable exceptions; enteroviruses and adenoviruses may be shed in the feces for weeks to months, and congenitally acquired CMV is shed in the urine for prolonged periods. Some factors that influence the likelihood of successful virus identification include the type of virus, the site from which the sample was obtained, the test being used, the age of the patient being sampled (e.g., younger children shed influenza viruses longer than adults), and the immune competence of the patient (e.g., immunocompromised hosts shed HSV from genital lesions longer than do immunocompetent adults) [12].

A serum sample should be collected early in the course of illness for potential use in identification of a viral infection. For some viral infections, the identification of IgM antibody or the presence of high titers of antibody is sufficient to confirm a virus infection. A second, or convalescent, serum sample should be obtained 2–4 weeks later to look for a rise in virus-specific antibody titer.

4.3 Clinical Data

Clinical information may be useful in helping one choose the types of diagnostic assays that should be performed on a clinical specimen. The time of year and age of the patient are examples of epidemiologic information that will influence the likelihood of identifying certain viral infections. For example, rotavirus infections occur seasonally and are more common in young children, while norovirus infections often occur in outbreaks and are more common in older individuals. Enteroviruses are the most common cause of viral meningitis and tend to occur seasonally in epidemics, whereas HSV type 1 is the most common cause of sporadic viral meningoencephalitis. Knowledge of the pertinent epidemiologic information will permit the use of appropriate enzyme immunoassays (EIAs), immunofluorescence assays, and other diagnostic methods to identify a potential viral pathogen in the clinical specimen.

5 Detection of Viral Antigens

The detection of viruses or viral components is the foundation of diagnostic virology. Although the detection of antibodies to specific viral proteins remains an important element of viral epidemiology, the ability to isolate and/or characterize viral pathogens initially is critically important. The performance of any diagnostic test in a reproducible, sensitive, and specific manner is crucial in the study of viral diseases. Combinations of various techniques, including centrifugation-enhanced tissue culture, antibody–antigen detection, and detection of viral nucleic acid, can be used to supplement classic tissue culture methods.

Methods for the detection of virus-specific antigens have allowed rapid identification of a wide variety of viruses (Table 3.1). Specific monoclonal antibodies conjugated to biochemical markers may provide high levels of sensitivity and specificity [13]. The key to the success of the assays outlined below is the use of reliable virus-specific antibody. Molecular biological techniques that permit the production of relatively large quantities of avid monoclonal antibodies have facilitated viral antigen detection.

5.1 Latex Agglutination Techniques

Viral-specific antibodies linked to latex beads can be used to detect viral antigens in a clinical sample. The presence of viral antigen in the sample results in cross-linking of the beads that can be identified by visual inspection. This

Table 3.1 Antigen detection methods used for diagnosis by virus group

Virus group	Immunofluorescence	Enzyme immunoassay	Immunohistochemistry
Enteric			
Adenovirus		++	
Astrovirus		+	
Norovirus		+	
Rotavirus		++	
Respiratory			
Adenovirus	++	++	+
Coronavirus	+		+
Human metapneumovirus	++	++	+
Influenza	++	++	+
Parainfluenza	++	++	+
Respiratory syncytial virus	++	++	+
Herpes viruses			
Cytomegalovirus	++		+
Epstein–Barr virus			+
Herpes simplex	++	+	
Human herpesvirus 8			+
Varicella–zoster virus	++		
Others			
Arenavirus		++	
Filovirus		++	+
Hantavirus			+
Hepatitis B virus		++	
Hepatitis D virus			+
Human papillomavirus			+
Rabies	++		+
Rubeola			+

+ occasionally used, ++ commonly used

strategy has most commonly been applied to detection of viral enteric pathogens like rotavirus and adenovirus, and it has the advantage of low complexity and providing rapid results (in less than 15 min). However, a major disadvantage is its relatively lower sensitivity (<70 %) compared to other antigen detection methods (>80–90 %) or culture [14, 15].

5.2 Immunofluorescence Techniques

Viral-specific antibodies conjugated to a fluorescein-labeled moiety have been used to identify viral pathogens since the late 1950s [16]. Immunofluorescence (IF) assays are widely used for the rapid detection of viruses in clinical samples and for definitive identification of a virus in tissue culture that may allow viral antigens to be sought before or after cytopathic effect (CPE) is evident. In the direct IF test (Fig. 3.1), the virus-specific antibody labeled with a fluorescent dye such as fluorescein isothiocyanate or, less commonly, rhodamine isothiocyanate is allowed to react for a short time with cells obtained from a clinical specimen or from an inoculated cell culture. After allowing time for an antigen–antibody reaction to occur, the slides are washed and examined microscopically for direct visualization of the fluorescence of the infected cells in the specimen. In the indirect IF test, two different antisera are used: an unlabeled virus-specific antibody capable of binding to a specific viral antigen is used first and is followed by a fluorescein-labeled, species-specific antibody directed against the species in which the first antibody was raised. If a reaction occurs between the first antiserum and the clinical specimen, the second antibody will bind to the antigen–antibody complex and fluorescence of the virus-infected cells can be detected.

The two IF methods each have advantages and disadvantages. Both tests allow an assessment of the quality of the sample in that samples that do not contain cells are poor quality and cannot be interpreted. The indirect IF is usually more sensitive because several fluorescein-conjugated molecules potentially are able to bind to each virus-specific antibody molecule attached to the viral antigen, resulting in amplification of the fluorescence. The direct IF test may offer enhanced specificity due to lower background fluorescence. The indirect IF method requires more reagents and more time to perform. Whether monoclonal or polyclonal antisera are optimal for use in either test method is still debated. The use of monoclonal antibodies generally

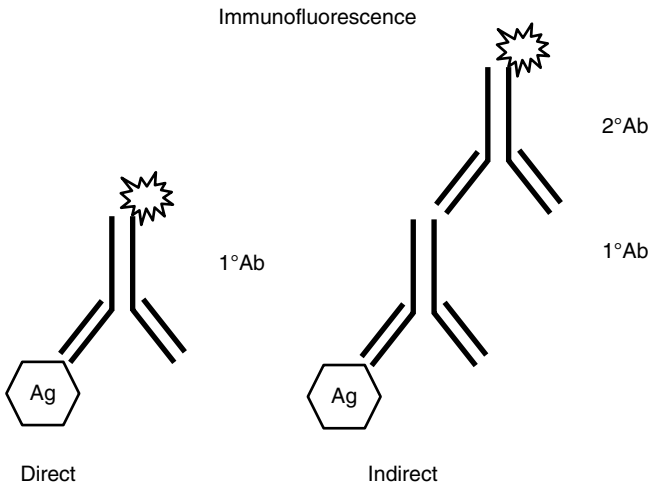

Fig. 3.1 Schematic of immunofluorescence. *Ag* antigen, *Ab* antibody

provides the lowest background but may be limited by the high specificity of these reactions. This problem can usually be overcome by using a pool of monoclonal antibodies.

The use of IF for the direct detection of viral antigens in clinical samples and the confirmation of viral growth in cell cultures has increased with the widespread commercial availability of relatively inexpensive antibodies specific for many of the herpesviruses and respiratory viruses. The IF method has the advantage of allowing rapid viral diagnosis in properly obtained specimens [17–20]. When working with large numbers of clinical specimens, the time required for sample collection, processing, and interpretation of the stained slide becomes substantial. The enthusiasm for this technique in clinical specimens has varied due to the time and degree of technical competence required to read such samples, the availability of other, the less labor-intensive antigen detection methods, and the frequency of false-negative results obtained because of the dependence of the assay on having a high degree of viral antigen expression in the clinical sample. Nevertheless, the appropriate use of this test can result in reliable and sensitive rapid diagnosis from clinical samples. The use of IF for the detection of RSV in pediatric patients by an experienced laboratory can detect up to 90–95 % of the samples positive by culture [21, 22], although many laboratories report rates of 60–80 % [23–25].

The combination of IF techniques with cell culture has increased the sensitivity of cell culture while providing a positive result in a shorter time period. With the use of centrifugation or other methods of enhancement of viral replication and pools of varying antibodies, cell cultures can be incubated between 1 and 3 days and then stained for a variety of virus antigens using indirect or direct IF methods. For some viruses, such as CMV or VZV, specific antibodies directed toward early or nonstructural antigens permit the rapid diagnosis within 48 h, well before CPE would be visualized under routine cell culture conditions [26, 27]. Disadvantages of IF techniques include the need for fluorescent microscopes, difficulty in the interpretation of clinical specimens that have a high level of nonspecific fluorescence, and the fact that prepared slides are not generally stable over periods longer than 1 month [20].

5.3 Immunocytochemical Staining

Immunocytochemical staining is a sensitive and specific method for detecting viral antigens with labeled antibodies. This technique, pioneered by Coons [28] in 1942, has been used to study the structure and function of a variety of viral proteins and continues to be utilized in both the research and clinical laboratories. It has been used both for detection of viral infection of a monolayer prior to the appearance of cytopathic effect and in rapid screening assays for drug resistance [29, 30]. This method utilizes reagents similar to those used in the IF assay except that the fluorescent marker is replaced by an enzyme. When enzyme-specific substrates are provided, a colored precipitate forms at the site of reaction. Typical enzymes used to detect viral antigens include alkaline phosphatase and horseradish peroxidase. A major drawback of alkaline phosphatase-based reagents is their lack of stability; a major drawback of peroxidase as a marker is the fact that this enzyme is endogenous to some mammalian tissue, thus requiring either elimination of the endogenous enzyme or use of a nonmammalian enzyme, glucose oxidase [31].

Advantages of immunoenzymatic staining compared to IF staining include the virtual permanence of stained preparations and the ability to view slides using an ordinary light microscope. Both direct and indirect staining with immunoperoxidase and other enzymes have been utilized to detect many viruses. Refinements have been developed that allow even greater sensitivity than that seen with indirect staining without the need to conjugate enzyme to an antibody. For example, a modification of these techniques has been a four-layer sandwich technique involving (1) virus-specific antibody raised in species X, (2) an excess amount of a second antibody raised against the species X antibody, (3) a complex of peroxidase and antiperoxidase antibody (raised in species X), and (4) reducing substrate for peroxidase [32]. The second antibody acts as a bridge, binding to both the virus and the antiperoxidase antibody. Similar unlabeled assay methods have been described for alkaline phosphatase–antialkaline phosphatase and glucose oxidase–antiglucose oxidase [33, 34]. The sensitivity of antigen detection has been further improved by more recently developed signal amplification methods, including avidin–biotin complexes (binding of 4 biotins per streptavidin), chain polymer-conjugated technology where multiple enzyme and antibody molecules are

attached to an inert molecule such as dextran, and the use of tyramine conjugates as substrates for horseradish peroxidase that allow signal amplication as much as 100-fold [35].

5.4 Radioimmunoassay

Radioimmunoassay (RIA) techniques have been valuable for the detection of many compounds in laboratories and clinical medicine. Initially, the technique was developed for the determination of endogenous human plasma insulin levels [4]. The first important use of RIA in diagnostic virology was for the detection of hepatitis B surface antigen [5]. The original RIA described by Yalow and Berson [36] was a competitive binding assay in which the competition between an unlabeled antigen and a radiolabeled antigen reacting with a limited amount of antibody over a short period of time was monitored. Variations in RIA methods have been developed, with the most common being the direct and indirect solid-phase RIA. In the direct solid-phase RIA, antigen or antibody are captured on a solid support and detected by radiolabeled (usually ^{125}I) antibody or antigen, respectively. The amount of signal increases proportionally to the amount of antigen or antibody present in the sample. In indirect assays, the capture of antigen or antibody to the solid phase prevents the binding of labeled antibody or antigen, respectively, so that the amount of signal detected is inversely proportional to the amount of antigen or antibody present. RIA methods currently are utilized mainly for the detection of antigens and antibodies of viral hepatitis [37]. The use of RIA for the detection of various hepatitis markers has demonstrated the assay's high degree of sensitivity. For the most part, RIA methods have been replaced by EIA for routine diagnostic purposes due to the complexity of the assay, the use of radioisotopes, lack of standardized commercially available reagents, and high equipment costs.

5.5 EIA

Enzyme immunoassays, or EIAs, have gained widespread acceptance in virology laboratories for the detection of a variety of viral antigens and antibodies. The assays used in this method rely on antibodies directed against a specific virus or viral antigen that are adsorbed or directly linked to polystyrene wells in microtiter plates, plastic beads, or membrane-bound material. When viral antigen is present in a specimen, it binds to the immobilized "capture" antibody and a second "detecting" antibody conjugated to an enzyme such as horseradish peroxidase or alkaline phosphatase then attaches to the antigen, forming a three-layer "sandwich" consisting of the immobilized antibody, the antigen, and the detecting antibody with enzyme attached (Fig. 3.2). A substrate

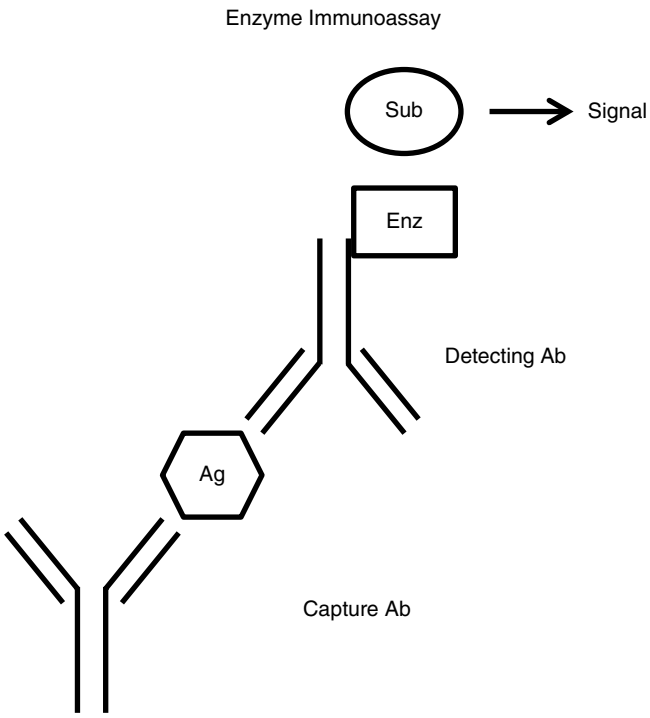

Fig. 3.2 Schematic of sandwich enzyme immunoassay. *Ag* antigen, *Ab* antibody, *Enz* enzyme, *Sub* substrate

specific for the enzyme is added and a color reaction occurs that can be monitored by spectrophotometry or by direct visualization. The test is quite simple to run, requiring only standardization of reagents and techniques such as dilution, incubation, and washing. The principles involved in EIA are similar to those involved in immunofluorescence and RIA, but the EIA test has the distinct advantages of being simple to perform, utilizing reagents that have long shelf lives, are inexpensive, and do not require sophisticated technical evaluation to determine results. Advantages of the EIA technique also include sensitivity (less than 1 ng/ml), specificity, rapidity, safety, automation potential, and low cost, particularly when many specimens require evaluation.

Variations in the methodology for EIA testing include the materials used, the procedures for incubation and detection, and the interpretation of results. Many different test kits are commercially available and in widespread clinical use for the detection of common viral pathogens such as RSV, influenza, adenovirus, HIV, norovirus, and rotavirus; EIA tests have been devised and reported for nearly all virus groups and continue to be used widely for clinical and research purposes.

5.6 Optical Immunoassay (OIA)

The OIA utilizes a virus-specific antibody coated onto a thin molecular film on a silicon wafer surface. The clinical sample

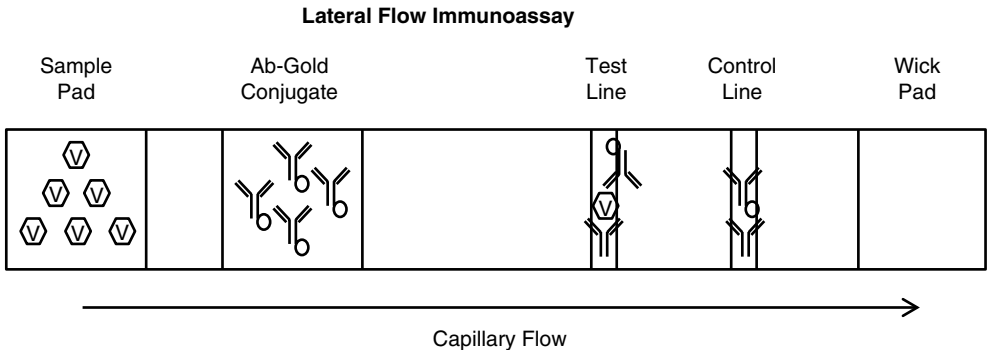

Fig. 3.3 Schematic of lateral flow immunoassay. *V* viral antigen, *Ab* antibody

is treated to extract and expose any viral antigens present and is then placed on the surface of the chip. Viral antigen is captured and the resulting antigen–antibody complex changes the optical thickness of the film on the chip. The change in the surface thickness is magnified through addition of a second virus-specific antibody conjugated to horseradish peroxidase followed by addition of a substrate such as tetramethylbenzidine (TMB). The presence of virus antigen is then detected by a change in the color of reflected light from gold to purple. Kits have been developed for influenza and respiratory syncytial virus detection [38, 39].

5.7 Lateral Flow Immunoassay

The lateral flow immunoassay, also called the immunochromatographic assay, is an immunoassay that is performed on chromatographic paper along a single axis (Fig. 3.3). The clinical sample is applied to an absorbent pad and then is drawn by capillary action through a conjugate pad. If viral antigen is present in the clinical sample, it will interact with a virus-specific antibody conjugated to a colored particle (often colloidal gold). The fluid in the sample carries the antigen–antibody complex to a reaction membrane to which another virus-specific antibody has been immobilized in a line perpendicular to the capillary flow direction. The antigen–antibody–conjugate complex is captured and can be observed as a colored line on the membrane. The sample is carried further across the reaction membrane to a control line. Antibody specific for the antibody–conjugate is immobilized along the control line, and visualization of the control line indicates that the sample migrated across the membrane and picked up the antibody–conjugate as designed. An absorbent pad is beyond the reaction pad and acts as a waste reservoir, drawing the clinical sample across the other pads by capillary action.

The simplicity of the lateral flow immunoassay design allows the use of these assays as point-of-care tests. Results can usually be obtained within 15 min of sample collection. Tests have been developed for detection of respiratory and enteric virus as well as dengue viruses [40–43].

5.8 Time-Resolved Fluoroimmunoassay (TR-FIA)

The TR-FIA is an immunoassay that replaces the reporter molecule with a lanthanide metal. When exposed to the appropriate wavelength of light, the lanthanide will fluoresce [44]. Compared to fluorescein and background autofluorescence, which have fluorescence decay times of less than five nanoseconds, the lanthanides have much longer decay times of 1,000 to 1 million nanoseconds [8]. The format of the antigen detection TR-FIA is similar to that of a sandwich EIA, where a microtiter plate is coated with a virus-specific capture antibody and is then blocked. The clinical sample and antibody conjugated to the lanthanide is added next, and after a suitable incubation period, the unbound components are removed by washing. An enhancement solution is added and the well is exposed to the appropriate wavelength of light. A fluorometer is used to measure fluorescence for 1 s, and the pattern of fluorescence allows the separation of antigen-specific signal from background fluorescence. Several different lanthanides are available for use, but europium is frequently used because of its long fluorescence decay time and the difference between its excitation wavelength (~340–360 nm) and emission wavelength (~615 nm) [8]. TR-FIA has been developed for detection of a variety of viral pathogens [45].

6 Laboratory Methods for Virus Characterization

Further characterization of a virus obtained from a clinical specimen is frequently desirable once an agent has been isolated. This can be done in a variety of ways, depending on what is known about the virus and what additional information is being sought. For example, if a previously unrecognized virus is recovered, characterization of its physicochemical as well as biological, antigenic, and genomic properties would be useful. Various immunological methods can be used for this purpose because of the general availability of immune reagents for most human viruses. Immunofluorescence,

radioimmunoassay, and enzyme immunoassay formats may be used in a fashion similar to that described for the virus detection in clinical specimens (Sect. 5). Other methods for virus identification and characterization include virus neutralization assays, hemagglutination-inhibition assays, and epitope-blocking enzyme immunoassays using monoclonal antibodies.

6.1 Neutralization Assays

Virus neutralization assays detect the loss of virus infectivity that results from the interaction of virus with specific antibody. Unknown viruses may be identified using virus-specific antisera, and antibody to a specific virus present in a serum sample can be detected or quantitated (see Sect. 7.1). The loss of infectivity can be measured in a number of ways, depending on the biological systems capable of supporting virus growth, the types of viruses being sought, and the capabilities of the laboratory performing the studies. The principal biological systems used for neutralization assays are tissue culture, embryonated chicken eggs, and adult and suckling mice [46]. Cell culture systems are used most commonly because they support the growth of a large number of viruses, are widely available, are easier to work with than the other two systems, and lack an immune system (that may influence test results). Embryonated hen's eggs and mice are used as for primary isolation. Neutralization cannot be measured for some viruses (e.g., norovirus) because their infectivity cannot be measured in currently available culture systems.

Pools of virus-specific antisera have been used to decrease the number of neutralization assays needed to serotype enteroviruses [47]. Each serum pool contains antisera to a discrete number of enteroviruses, and antiserum to a given enterovirus is present in one to three pools. Thus, the pattern of neutralization obtained from the use of only eight intersecting serum pools allows the identification of 42 different enteroviruses [48]. Methods for the production of intersecting serum pools have been published [49].

6.2 Hemagglutination and Hemagglutination-Inhibition Assays

The ability to agglutinate erythrocytes, a property shared by many viruses, can be used for the identification of some of these viruses. The differential hemagglutination of rat, human group O, and monkey erythrocytes by different adenovirus serotypes allows their separation into groups so that fewer type-specific sera need to be used in neutralization or hemagglutination-inhibition assays [50]. Type-specific antisera can be used to prevent hemagglutination (hemagglutination inhibition) and permit the identification of influenza A and B viruses, parainfluenza viruses, and adenoviruses [51].

6.3 Agar Gel Immunodiffusion

Agar gel immunodiffusion, or agar gel precipitation, has been used for the characterization of a variety of viral antigens using standard, or reference, antisera. A thin layer of agarose is made in a plate or on a slide, and small wells are cut into the agarose. The unknown antigen and known antiserum are placed in separate wells, and the proteins in the wells diffuse through the agarose. If the antiserum reacts with the virus antigen, a precipitation band appears. Though less sensitive than other methods and largely replaced by molecular assays, this method offers high specificity and is simple to perform. It has been used for the identification of orthopoxviruses, typing of influenza viruses, and subtyping of hepatitis B viruses [51–53]. It also has been used to characterize unknown sera with known virus antigens [54].

6.4 Antigenic Characterization

The antigenic differences or similarities between vaccine and wild-type strains or among virus strains that have been isolated from different geographic locations or at different times may be examined in a number of ways. The availability of monoclonal antibodies permits the examination of these relationships and may detect differences or similarities that cannot be detected by polyclonal antisera [55, 56]. These assays examine the ability of a given monoclonal antibody to interact with a particular virus strain and are performed using the same formats used for polyclonal antisera: RIA or EIA, neutralization (if antibody is neutralizing), immunoprecipitation, hemagglutination inhibition (if the virus has hemagglutination activity), and so forth.

Monoclonal antibodies also have been used to map antigenic sites on virus proteins. When a virus is grown in the presence of a monoclonal antibody that normally neutralizes it, the only progeny virus will be escape mutants, or viruses that are no longer neutralized by the antibody. Frequently, escape mutants arise after substitution of a single nucleotide, resulting in a single amino acid change, and the location of the change can be determined by sequencing the virus gene(s) encoding the viral protein(s) important in neutralization (e.g., rotavirus) [57, 58]. A less precise map of antigenic sites can be obtained through the use of a panel of monoclonal antibodies by determining whether an individual monoclonal antibody competes with other monoclones for an antigenic site and whether the antibody has activity against the escape mutants raised by a different monoclonal antibody [59].

7 Serological Diagnosis

The detection of newly developed, virus-specific antibody or the detection of an increase in titer of preexisting antibody is important in viral diagnosis and is one of the most commonly used methods in epidemiologic studies of viruses. Most primary infections or reinfections result in the production of specific antibodies. In addition, viruses such as EBV, HIV, rubella, hepatitis A and B viruses, and arboviruses are difficult to detect directly and the serological diagnosis may be the only practical means of identifying the particular agent.

The detection of specific IgM antibody may be used to suggest a recent infection in a single serum specimen. Detection of specific IgM antibody in the neonate is useful to diagnose congenital infections, because maternal IgM antibody does not cross the placenta. IgM antibody also is useful to detect acute disease in a variety of other clinical situations, including infection with CMV, rubella, hepatitis A and B, and EBV. Limitations to the use of IgM detection include the following: (1) IgM-specific antibody is not restricted to primary infection and may be seen with reactivated disease, particularly with herpesviruses such as HSV or varicella–zoster; (2) false-positive responses may occur in the presence of rheumatoid factor or false-negative results from competition by IgG antibody for binding sites on the antigen; (3) IgM antibody may persist for months to a year or more after an infection occurred; and (4) heterotypic reactivation of IgM may be found with some infections (such as CMV or EBV). For example, removal of Coombs antibody from sera is necessary for the EBV–VCA–IgM test; otherwise, false-positive results may arise. Methods useful for the detection of viral-specific IgM will be described below, but, in general, diagnosis using a single IgM sample needs to be carefully controlled to exclude the detection of IgG or other interfering substances.

Many different serological techniques have been used in the diagnosis of viral infections (Table 3.2). Factors involved in the selection of a specific antibody assay include specificity, sensitivity, speed, technical complexity, cost, and availability of reagents (Table 3.3). All antibody assays rely on the proper collection and storage of sera and, ideally, the comparison of acute and convalescent specimens collected at an interval of at least 2 weeks. The development of newer techniques, such as EIA, for antibody determination is replacing some of the older methods, such as complement fixation, but an understanding of the available methods is important prior to choosing a laboratory test to evaluate a specific question.

Table 3.2 Serological diagnosis of detected viruses

	Serological method[a]			
Virus	Neutralization	Complement fixation	Hemagglutination inhibition	Immunoassay (EIA, IF)
Adenoviruses	+	+		+
Arboviruses	+	+	+	++
Coronaviruses	+		+	+
Cytomegalovirus	+	+[b]		++
Enteroviruses	+		+	
EBV[c]				++
Hepatitis B and C				++
HSV	+	+[b]		++
Influenza	+	+[b]	++	+
Measles	+	+[b]	+	++
Mumps	+			++
Norovirus/rotavirus			+[d]	++
Parainfluenza	+	+[b]	++	+
Parvovirus				+
Rabies	+			+
RSV	+	+[b]		++
Retroviruses	+			++[e]
Rhinoviruses	+			+
Rubella			+	++
VZV		+[b]		++

[a]+ Method used in research setting, ++ method in use and readily available in virology laboratories
[b]Complement fixation method may lack sensitivity for these viruses
[c]The absorbed heterophile test is commonly used for infectious mononucleosis, with the EBV–VCA–IgM needed if that test is negative
[d]Selected strains only
[e]Western blot commonly used as confirmatory test

Table 3.3 Comparison of serological methods used to detect viral antibodies

Method	Sensitivity[a]	Specificity	Cost	Time to Dx	Availability
Neutralization	+++	++	Expensive	>1 week	Research
Complement fixation	+	+/++	Inexpensive	<1 day	Widely available
Hemagglutination inhibition	++	++	Inexpensive	<1 day	Research/reference labs
Enzyme immunoassay	+++	++	Inexpensive	<1 day	Widely available
Immunofluorescence	++/+++	++	Moderate	<1 day	Research/reference labs
Radioimmunoassay	+++	++	Expensive	1–3 days	Research
Immunoblot (Western blot)	++/+++	+++	Expensive	<1–3 days	Research/reference labs

[a]+ relatively low, ++ moderate, +++ high

Problems specific to serodiagnosis of a viral infection include the broad cross-reactivity among some virus groups, such as the coxsackie A viruses that cross-react with antibodies to coxsackie B and echoviruses. Another serious limitation of this approach to diagnosis is the failure of some individuals, particularly young children or immunocompromised patients, to mount a detectable antibody response to a specific infection. However, serological methods remain extremely important in epidemiologic studies because results are not dependent on obtaining a specimen at the peak of illness, tests can be performed retrospectively for a variety of agents simultaneously, and large-scale studies can be conducted in a timely and cost-effective manner.

7.1 Neutralization

Serum specimens may be assayed for neutralizing antibody against a given virus by testing serial dilutions of the serum against a standard dose of the virus. The antibody titer is expressed as the highest serum dilution that neutralizes the test dose of virus. As a bioassay, neutralization assays are highly specific and quite sensitive. For many viral agents, the neutralizing antibody level is directly correlated with immunity, an important clinical and epidemiologic endpoint. Disadvantages of the assay include the time required to obtain a result and the relatively high cost, due to the labor intensity and requirement for cell culture and titered viral stocks. Neutralization assays may be carried out in a variety of systems and the endpoint measured by a number of different procedures. Different neutralization systems include plaque reduction neutralization, sometimes using complement enhancement, where the number of virus plaques in control wells are compared with the number seen in cultures inoculated with the virus–serum mixture; microneutralization, an assay performed in microtiter plates requiring small amounts of sera; and colorimetric assays. Colorimetric assays rely on markers indicating metabolic inhibition of the virus in cell cultures or on antigen–antibody reactions with antibody tagged or reacted with enzyme-linked antibodies. Colorimetric assays are generally analyzed by measurement of optical density and have the advantage of being less time-consuming and costly to set up and analyze than other assays. Colorimetric assays often are more sensitive than assays relying on inhibition of CPE [60].

The type of assay used to assess antibody is very important, particularly in the evaluation of susceptibility to vaccine-preventable or epidemic viruses such as measles or rubella, where low levels of antibody may be predictive of protection. Direct comparison of various laboratory methods used for the detection of virus-specific antibody may be important in designing studies or evaluating study results. For example, analysis of CMV antibody using neutralization by plaque reduction has shown poor correlation with CMV antibody using EIA [61]. Different neutralization methods also may give differing results, as has been shown in the analysis of antibody to RSV, where microneutralization assays appear to be more indicative of biological protection than either direct or competitive ELISA methods or complement-enhanced plaque reduction [62].

In general, measurement of neutralizing antibody is the most specific method that reflects immunity, although other tests for some viruses may be surrogate markers for this. However, neutralization tests are rather expensive since a demonstration of inhibition of viral replication in cell culture, embryonated egg, or laboratory animal (such as the suckling mouse) is required.

7.2 Complement Fixation

The complement fixation (CF) test is a relatively simple technique that may be used successfully with a large variety of viral antigens. First developed in 1909 by Wasserman and coworkers [63, 64] for the detection of syphilis antibodies, CF has been adapted to test for antibodies to many bacterial and fungal pathogens of animals and man. The CF test relies on competition between two antigen–antibody systems for a fixed amount of complement, with the result ultimately demonstrated by the lysis of erythrocytes. The serum is heated at 50 °C for 30 min to inactivate any complement that may be present. Antigen and a known amount of complement are added to dilutions of serum. The complexes formed between the initial antigen and antibody bind the available free complement, thus preventing further reaction of the complement in the second step of the assay. In the second step, a

hemolytic indicator system using red blood cells (RBCs), which have been reacted with hemolysin to sensitize them to complement, is used to detect the free complement. The RBCs are reacted with hemolysin, or antibody to the RBC, and added to the assay. Lysis of the RBCs occurs if free complement is present. Thus, the presence of hemolysis at the conclusion of the assay is indicative of the absence of specific antibody, whereas the formation of clumped RBC (often referred to as a "RBC button") indicates a positive test reaction. Antibody titers can be calculated using standard endpoint determinations. Antibodies detected by CF are primarily of the IgG class and develop during the convalescent stages of illness. The greatest application of the CF test lies in the demonstration of a rise in antibody in convalescent compared with acute sera. The CF test has been widely used for the serodiagnosis of many human viral pathogens because of its broad reactivity and effectiveness in detecting changes in antibody titers and it is often the standard against which new methods are compared. The CF test has largely been replaced in many laboratories by enzyme immunoassays, but it continues to maintain its usefulness in some circumstances because reagents are relatively inexpensive and readily available and the test is rapid, reliable, and relatively easy to perform [65]. Another advantage of this assay is that many antigens can be easily tested in the same sera samples simply by changing the antigen but keeping all other reagents and conditions the same. The CF test is also adaptable to microtiter and automated methods.

Despite the widespread use of the CF assay over time and in many epidemiologic studies, the assay has some unique problems: (1) the test relies on a cascade of interactions involving multiple biological reagents that must be carefully monitored; (2) it is relatively insensitive because high concentrations of antigen are required to produce CF complexes and the assay is unable to detect small changes in antibody concentrations or low levels of antibody that may be predictive of protection in other assay systems (such as VZV or measles) [66, 67]; and (3) sera containing antibodies to host cell components or anti-complementary sera will not give a valid result. Newer laboratory methods, such as EIA methods, are now available commercially and have largely replaced CF tests for many viral pathogens because of their ability to discriminate between IgG and IgM antibody, increased sensitivity, and enhanced specificity at a similar cost.

7.3 Hemagglutination Inhibition

The hemagglutination-inhibition test (HAI) is based on the ability of some viruses to attach to receptors on certain species of erythrocytes and cause hemagglutination (Sect. 6.2). While HAI may be used to identify an unknown virus with virus-specific antisera, it also is useful for the detection of virus-specific antibodies in the serum. HAI may be used to evaluate antibody titers of influenza viruses, parainfluenza viruses, adenoviruses, rubella, arboviruses, and some strains of picornaviruses and noroviruses [68, 69]. In this assay, serial dilutions of sera are allowed to react with a defined amount (4 HA units) of viral hemagglutinin. Subsequently, agglutinable RBCs are added and the ability of the virus to agglutinate the RBCs is measured. Properly treated sera containing antibody specific to the virus will prevent agglutination of the RBCs, resulting in formation of RBC buttons in the test wells; sera lacking specific antibody will result in RBC agglutination. Nonspecific viral inhibitors can give rise to false-positive results in some systems, requiring that the sera be properly prepared prior to use. The specificity of the assay varies somewhat with the particular virus, with influenza and parainfluenza virus systems being more specific than the arbovirus system [69]. For example, the HAI test can identify specific strains of influenza viruses, whereas it identifies only the group-specific antigens of the arboviruses (with neutralization tests required for strain identification). Advantages of the HAI assay are its simplicity, the low cost for reagents and equipment, and speed of the assay. Disadvantages of this assay include the fact that the system only works with those viruses that cause hemagglutination and that nonviral-specific serum components may also inhibit hemagglutination, thereby invalidating the test results.

The immune adherence hemagglutination method is another method that has been used in the clinical laboratory. After an initial reaction between viral antigens and specific antibodies is allowed to occur, complement is added and binds to the antigen–antibody complex, if present. Human erythrocytes then are added and reaction to the antigen–antibody–complement complex with the C3b receptor causes hemagglutination. This method, commonly used as a microtiter procedure, has a well-defined endpoint and is rapid, inexpensive, and more sensitive than CF [70]. Disadvantages of this method include the inability to differentiate between different immunoglobulin subclasses and its difficulties with viruses that themselves agglutinate RBCs.

The direct agglutination of sheep or horse RBCs by serum is a diagnostic test for the heterophile antibody of infectious mononucleosis. Removal of an inhibitory (Forssman) antibody by adsorption of the sera with guinea pig kidney extracts is required before testing. The hemolysis of beef cells by sera from patients with acute infectious mononucleosis due to EBV is another diagnostic test that does not require adsorption but is less sensitive (see Chap. 37).

Red blood cells or latex particles can be coated with viral antigens and used to determine the presence or absence of viral antibodies in reactions called passive hemagglutination and passive latex agglutination, respectively. If viral antibodies are present, the antigen-coated red blood cells or latex particles are agglutinated. This assay is subject to a prozone effect, in which an excess of antibody reduces or eliminates agglutination leading to a false-negative result. To circumvent

this problem, negative samples can be diluted and the assay repeated. Commercial kits are available for the qualitative identification of antibodies to VZV and rubella virus [71].

7.4 Immunoassay Techniques

7.4.1 Enzyme Immunoassay

Enzyme-based immunoassays (EIAs), sometimes called enzyme-linked immunosorbent assays or ELISA, are widely used for many purposes, including the detection of antigen-specific antibody. This methodology has replaced other methods in many laboratories in part due to the commercial availability of test materials and complete test kits. The most commonly used immunoassays employ a four-layer approach: antigen is bound directly to a surface, the unknown serum sample is then added, an enzyme-conjugated antihuman IgG or IgM is added next, and an indicator system is used to determine the amount of reaction between the enzyme-linked antibody and the antigen–serum reaction. As discussed in Sect. 5.5, this method has gained widespread use due to its sensitivity, specificity, safety, simplicity, low cost, and ability to be automated. The system lends itself to automation because of readily available microtiter diagnostic systems and because multiple tests for different antigens may be run by varying only the initial antigen in the system. Clinical specimens from various sources besides serum or plasma, such as respiratory secretions, cerebrospinal fluid, and breast milk, also may be tested in this system. The EIA test may also be read visually and so is useful in developing countries unable to afford the photometric reader used in developed countries to accurately quantitate the antibody content.

Difficulties inherent in immunoassay techniques include those associated with obtaining and standardizing purified, sensitive, and specific IgG and IgM reagents. Although many of these reagents are commercially available, there can be variability in the specificity of the reagents so that during assay development the reagent specificity should be assessed. In general, results from different laboratories using different reagents are not directly comparable. Specific analysis for subclasses, particularly IgM, requires careful standardization and quality control to assure reliability and specificity of the assay. Attention to such detail is critically important in assays with life-threatening implications, such as the EIA assays currently used in blood banks to detect the presence of antibody to HIV or hepatitis B and C. Evaluation and standardization of tests, including commercially available kits, and comparison among different products prior to use in research and clinical settings remain an important part of EIA.

The lateral flow immunoassay described in Sect. 5.7 can be modified to detect viral antibody. Antihuman immunoglobulin or protein A labeled with conjugate is used in the reagent pad in place of viral antibody conjugates, and the labeled conjugate can interact with virus-specific antibodies in the clinical sample. Recombinant viral antigen is used to capture the virus-specific antibody–antibody–conjugate complex in a "test" line, and a "control" line containing antihuman immunoglobulin is present to indicate that the assay performed as intended. The test format provides a rapid qualitative answer with sensitivity and specificity similar to that obtained by more complex laboratory-based enzyme immunoassays [71, 72]. These assays can be applied to serum as well as to saliva, and they have been developed for several different viruses including HSV, HIV, hepatitis C virus, and chikungunya virus [72–75].

Epitope-blocking assays using monoclonal antibodies have been used to examine serological responses to a number of viruses [76–81]. These assays measure the ability of a test serum to block the binding of a monoclonal antibody to a virus antigen. They have been particularly useful in determining serotype-specific immune responses to multivalent vaccines and in determining which of a number of cross-reactive virus strains is responsible for a natural infection in a given host.

7.4.2 Other Immunoassays

Other variations of the immunoassay include isoelectric focusing and affinity immunoblotting. These techniques are useful because of enhanced sensitivity, particularly for diagnosis in the congenitally infected infant or for the differentiation of passively acquired antibody from endogenous antibody. Isoelectric focusing relies on the separation of serum antibody in thin-layer gels, with subsequent antibody detection by a reaction with antigen-coated membranes [82]. Clonal-specific antibodies can be detected by this method, which may discriminate, for example, between unique maternal and fetal antibodies.

The TR-FIA assay can be modified for detection of virus-specific antibodies by coating the well of the microtiter plate with viral antigen and labeling antihuman immunoglobulin with the lanthanide reporter. The availability of lanthanides with distinct emission spectra allows multiplexing of the assay such that different antibody isotypes (e.g., IgG and IgA) can be measured in the same well [83, 84].

Radioimmunoassay techniques for the sensitive detection of antibody to viral antigens, pioneered in the 1970s for the serodiagnosis of hepatitis B, are used by research laboratories but otherwise are not widely utilized [5]. Immunoassays that do not require radioisotopes and require less technical expertise, such as the EIA and IF tests, are more commonly available.

7.4.3 Immunohistochemical

The most commonly used immunohistochemical technique to detect antibody is the immunofluorescence technique (IF). Whereas direct techniques are used commonly to detect

antigen in infected tissue or cells, indirect immunohistochemical techniques are used to detect antibody in sera or other bodily fluids. Variations on the indirect IF test, such as amplification immunoassay systems utilizing various sandwich techniques (double indirect IF or anticomplement IF) or chemical amplification systems utilizing biotin–avidin complexes, are used in research settings [65]. Indirect IF methods are available in many laboratories for a variety of assays, although more automated techniques such as EIA are replacing IF techniques in many clinical settings.

In the indirect IF test, tissue or cells containing viral antigen are fixed on a glass slide, a serum dilution is added, and a fluorochrome-conjugated antibody indicator system is used to detect the resulting reaction. The conjugated detector antibody can be varied to specifically measure the presence of antibody classes, such as IgG, IgM, and IgA. IF techniques provide sensitive methods to detect antibody that can be related to immunity to viruses, such as VZV, to detect congenital infections in newborns based on IgM-specific antibody, and to detect epitope-specific antibody [65, 85]. In particular, the anticomplement-amplified indirect IF technique, utilizing a four-layer reaction including antigen, patient serum, complement, and fluorochrome-conjugated anti-C3 antibody, has been useful for the detection of the nuclear antigen of EBV, or EBNA [65]. The inclusion of standard positive and negative sera is needed in each test and independent reading by two observers is recommended to minimize errors in interpretations.

Advantages of indirect immunohistochemical methods include (1) the ability to use the system to detect antibody to many diverse viruses by varying only the initial step in the reaction, (2) the higher sensitivity and specificity compared with CF, (3) the simplicity and relative speed of an individual test, and (4) the reproducibility of the test by experienced personnel. Disadvantages of the test include the technical complexity, the lack of automation, the requirement for specialized cells and reagents, and the need for special equipment, such as a fluorescence microscope and darkroom.

7.4.4 Immunoblot

An immunoblot, also referred as a Western blot, is another widely used method for the detection of antibody to specific viral antigens. This technique relies on the incubation of patient serum with partially purified whole virus or recombinant viral proteins that have been separated by electrophoresis in a polyacrylamide gel and transferred onto nitrocellulose paper [86]. The assay is based on the same principal as EIA but has the advantage of identifying antibodies specific for several antigens of the same virus simultaneously. Quantitation of the specific reactions can be determined by a densitometer. Difficulties with immunoblots include the expense and time required for the test, the technical requirements for performing and interpreting the test, and the problems encountered with preparing reagents such as purified virus and labeled antihuman IgG. The time interval from virus acquisition and seroconversion by immunoblot may vary for different patients, as well as different viruses, indicating that diagnosis of infection by immunoblot analysis, while extremely sensitive, is not always definitive [87]. Furthermore, analysis by immunoblot will not differentiate maternal from fetal HIV infection in many cases. Despite these problems, the immunoblot assay is widely used today in laboratories around the world as the "definitive" confirmatory test for HIV, and it is also used as a confirmatory test in some circumstances for the serological diagnosis of hepatitis C virus infection [88]. It also has been used to differentiate type-specific serological responses to herpes simplex virus type 1 (HSV-1) and HSV-2 [89].

8 Interpretation of Laboratory Tests

Virus infection is usually identified by either detection of the virus or identification of a serological response to the virus. For some viral infections, both are required. However, the interpretation of the viral diagnostic assays must be made in the context of the assay sensitivity and specificity along with seasonal, clinical, and other epidemiologic factors.

Difficulties arise in interpretation of serological tests when only a single convalescent serum sample is obtained and a high antibody titer is found or if high titers are present in both acute and convalescent sera without a fourfold rise. These results can reflect either current infection or persistently high antibody levels from a previous infection. Significance may be attached to these findings if the disease is a rare one in which the presence of antibody is unique, if the test reflects a short-lasting antibody, or if specific immunoglobulin M (IgM) antibody can be demonstrated. A rapid drop in antibody titer in a subsequent specimen is also suggestive of a recent infection. Sequential testing of other family members also may be useful, since they may be in different stages of apparent or inapparent infection with the same virus. In an epidemic setting, comparison of the geometric mean antibody titer of sera collected early in illness from one group of patients with that from another group of patients convalescing from the same illness may permit rapid identification of the outbreak.

At times, a virus may be identified or an antibody rise demonstrated that is not, in fact, causally related to the illness. Sometimes dual infections with two viruses, or with a virus and a bacterium, occur, and the interpretation of the role of an individual viral pathogen in the disease process may be very difficult. On other occasions, no virus is isolated or the serological rise is not sufficient to demonstrate whether a specific virus is the real cause of the illness. A list of common causes for false-positive and false-negative results is given in Table 3.4.

Table 3.4 Viral diagnosis: some causes of false-positive and false-negative tests

False positive
Antigen detection
1. Persistent or reactivated virus from prior and unrelated infection
2. Two microbial agents are present, and the one isolated is not the cause of the disease
3. Mislabeled specimens
4. Cross-contamination within the laboratory
Serological rise
1. Cross-reacting antigens
2. Nonspecific inhibitors
3. Double infection with only one agent producing the illness
4. Rise to vaccination rather than natural infection
False negative
Antigen detection
1. Viral specimen taken too late or too early in illness
2. Wrong site of multiplication sampled, e.g., throat rather than rectal swab
3. Improper transport or storage of specimen
4. Low assay sensitivity
Serological rise
1. Specimens not taken at proper time, i.e., too late in illness or too close together to show antibody rise
2. Poor antibody response–low antigenicity of the virus or removal of antibody by immune-complex formation
3. Wrong virus or wrong virus strain used in the test
4. Nonspecific inhibitor obscures true antibody rise
5. Wrong test used for the timing of the serum specimens

9 Unresolved Problems

Many problems still must be resolved in the area of viral diagnosis. The development of newer molecular assays with improved sensitivity have supplanted many antigen detection methods with lower sensitivity, but the ease of performance of the antigen detection methods still makes these assays attractive to many laboratories. While molecular methodologies offer the potential for great sensitivity and specificity, they often require specialized equipment for their performance. Ultimately, the ideal diagnostic test will be rapid, easy to perform, inexpensive, sensitive, and specific and will not require the use of specialized equipment. However, even if such a test is developed, the significance of the identified virus to the observed disease process will remain in the hands of the epidemiologist, virologist, and clinician.

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