

Viral Immunodiagnosis

A. BAUMGARTEN

*Department of Laboratory Medicine, Yale University School of Medicine,
New Haven, Connecticut*

Received August 6, 1979

This review discusses the commonly employed methods for viral immunodiagnosis, mentions unusual or novel procedures, and briefly refers to the use of monoclonal antibody.

INTRODUCTION

The perception of the spectrum of viral disease has expanded markedly owing to a recognition of the role of viruses in such diverse states as juvenile diarrhea, congenital mental impairment, death after transplantation, cancer, and chronic neurological dysfunction. The transmission of viral disease has been enormously facilitated by widespread air travel which has placed even such exotic horrors as the lassa fever on the doorstep of potentially any physician. Meanwhile, the isolation of interferons and the prospect of effective chemotherapy have further accentuated the need for prompt and effective viral diagnostic measures. Fortunately, this requirement has been accompanied by the growth of an increasingly sophisticated methodology of immunoassay.

Immunodiagnostic assays can rapidly identify the virus in cell smears (e.g., buccal, cervical) or body fluids (e.g., CSF, urine) as well as determine the class and concentration of antibody to a selected antigen (e.g., early or late) in any body fluid. The assay of antigen is familiar for hepatitis. It may prove equally familiar for cytomegalovirus, herpesvirus 2, and others with the development of effective measures for the control of the viruses. The assays for humoral and cellular immunity are becoming increasingly valuable as the significance of the various modalities of the immune response is recognized for its diagnostic and prognostic implications. The assays are also likely to become important for monitoring the efficacy of vaccination and chemotherapy.

The exploitation of immunoassay for viral diagnosis supplements the standard procedures of culture, complement fixation, electron microscopy, etc., which are frequently slower, less sensitive, or more expensive. The newer procedures offer specialized alternatives to the dedicated viral laboratory but, even more important, they bring viral immunodiagnosis within the reach of a competent hospital laboratory. The ambiance of an immediate availability of the tests in a clinical milieu should favor their effective utilization.

Consideration of viral immunodiagnosis can be undertaken under three headings: the virus as an antigen, the immune response to the viral antigen, and the methods of assay. This synopsis will deal principally with the last of the three topics.

TABLE I
Viral Antigens

<i>Virus associated</i>
External antigens
Functional:
—for attachment
infectious
adventitious
—enzymatic
Structural:
glycoproteins
other substances
Internal antigens
<i>Cell associated</i>
Early antigens
(formed before viral replication)
Late antigens
(formed after replication has begun)
<i>Immune complexes</i>

Virus as an Antigen

Table I lists the types of viral antigen. Identification of an infecting virus in the cells of the host, or in culture, generally depends on external antigens of the virus or on cell-associated antigens. Immunodiagnostic identification requires the availability of suitable, specific antisera. Subsequent characterization of the antigenic composition of a virus can utilize disrupted viruses or cells and employ internal antigens. The presence of the antigens can be assayed by general methods, such as complement fixation (CF), radioimmunoassay (RIA), or enzyme linked immunoassay (EIA), but an initial identification of antigens in a mixture is often best accomplished by precipitation techniques (for example, double immunodiffusion, immunoelectrophoresis, or crossed immunoelectrophoresis). External or cell-associated antigens are generally detected by methods such as immunofluorescence (IF) or EIA. Functional antigens responsible for viral attachment to cells are tested in neutralization assays, while adventitious attachment is assayed by hemagglutination. Glycoproteins, in addition to being identifiable by the various procedures used for structural antigens, may also be detected by specific reactivity with lectins. Inhibition of a viral infection by lectins has been described [1]. A systematic use of lectins for viral identification has not been attempted but seems to offer interesting possibilities.

The identification of cell-associated antigen has been useful chiefly in the assay of the appropriate antibodies. However, cell-associated antigens especially the early ones, can be detected with appropriate antisera in tissue or cultured cells for quicker identification of the virus.

Detection of immune complexes has become clinically significant [2], especially in patients with nephritis, vasculitis, or autoimmune disorders. Identification of the antigen in the complex assists in the differential diagnosis of the disorder. Furthermore, identification of the responsible virus permits a distinction to be drawn between direct consequences of an underlying disease (e.g., an autoimmune state) and a complicating viral infection. It also allows the detection of viral infections which are most likely to become complicated by sequelae caused by immune complexes.

Immune Response in Viral Infection

The immune response in viral infection includes both a cellular and a humoral component [3] but either, or both, may fail to occur in an immunodeficient or immunosuppressed individual. Manifestation of immunity may be systemic and/or local. Either of these manifestations can develop without the other.

Local immunity is characterized by an IgA antibody response which occurs typically within one to two weeks, varies in duration, but does not manifest a recall (secondary, booster, or memory) component. A variable amount of the antibody in secretion is derived from serum. This fraction can be increased if there is local bleeding, ulceration, or other cause for increased exudation. Clinical assessment of antiviral, secretory antibody has received only limited attention, but may be important for local recurrence or persistence of infection (e.g., in the respiratory [4], and urogenital tracts [5]).

Cell-mediated immunity is a prominent feature of the host's response to the virus. It was the first immune response to have been described, "a change produced in the constitution [which] is not affected by time" [6]. Recently, it has been shown that the ability of lymphocytes to respond to a viral infection depends on the recognition of a histocompatibility antigen [7] modified in the course of the infection, or co-present on the cell surface with a closely juxtaposed, virally determined antigen. Quantification of cell-mediated immunity is, however, subject to technical difficulties which have prevented its routine exploitation. Nevertheless, *in vitro* tests can provide substantial information about an individual's ability to respond to viral infection [8,9]. Further development of these tests may assist in the clinical analysis of virally induced, cell-associated antigens and be useful in the detection of recent or current infection [10], or differentiation of an infectious syndrome (e.g., mumps meningitis [11]). *In vivo* challenge (skin testing) with viral antigens is limited in usefulness. Its primary role is in the detection of an individual's ability to respond to common viral antigens as a measure of the retention of a competent cell-mediated immune response (delayed hypersensitivity) rather than as a diagnostic tool in infection [12].

An aspect of cellular immunity which may gain special prominence is the measurement of virally induced lymphokines (e.g., migration inhibitory factor [13] or interferon [14]). The measurement of interferon level in serum may be used either to assess the response to infection or to monitor the persistence of the material administered for therapy.

Humoral antibody can be used in two ways. Specific antibody can be employed to identify the virus recovered from an individual or present in tissue culture. Systemic antibody can be assayed to determine the type and course of infection by the use of viral antigen of known specificity.

The systemic antibody response can involve all classes of immunoglobulins [15]. Generally, an IgM response is observed within one to two weeks (e.g., with CMV, EBV, measles, rubella, mumps) and an IgG response a few days later [16]. A maximal IgM response is typically seen in three to six weeks, with subsequent decline in two to three months, while an IgG response becomes maximal in four to twelve weeks and persists for months or years before declining. Determination of the IgM response is therefore more useful in primary or current infection [17,18] and that of IgG for the assessment of immunity (e.g., to poliomyelitis) or epidemiological study, as well as in acute infection. However, an IgM response may not be apparent, may be intermittent or prolonged, or may occur later. It may recur with subsequent infection. Similarly,

TABLE 2
Features of Some Common Viral Infections

Infecting Agent	Typical Interval Before the Appearance of		
	Symptoms (days)	Antibody (weeks)	Immunodiagnostic Method(s)*
Adenovirus	4	2-3	CF, N
Cytomegalovirus	40	2-3	CF, IF
Enterovirus	9	1	N
Epstein-Barr Virus	35	1	HET, IF
Herpesvirus	6	<1-2	CF
Influenza	2	1-2	CF, HI
Measles	10	<1	HI, CF
Mumps	14	<1	CF
Rubella	17	1	HI, CF

*Abbreviations: CF = complement fixation
HI = hemagglutination inhibition
N = neutralization
HET = heterophile
IF = immunofluorescence

variation is encountered in the level and duration of the IgG response. These differences are reflected in the results of tests which measure the antibody. Furthermore, some assays are more affected by IgM antibody [19] (for example, passive or indirect hemagglutination, or hemagglutination-inhibition), while others depend on IgG (e.g., IgG-specific immunofluorescence), so that diverse results can be obtained. The lapse of time between infection and the appearance of measurable antibody also varies with the virus (Table 2) and even the viral antigen: e.g., with EBV the antibody to viral capsid antigen appears within days, to nuclear antigen after several weeks or months, but to soluble antibody (by complement fixation) after months to years [20].

Systemic antibody of the IgA or IgD class [15] has undetermined biological significance. Antibody of the IgE class [21] has also received little attention, possibly because viral disease is generally unassociated with immediate hypersensitivity to the virus, though allergy to vaccines can occur, especially to contaminants such as egg protein.

Methods for Immunodiagnosis

The methods used for immunodiagnosis employ either labelled or unlabelled reagents (Table 3). Application of the commonest methods is represented in Table 4. An example of a novel approach to the use of unlabelled reagents is calorimetry [22]. The results of the various methods are likely to differ owing to differences in their sensitivity as well as the specificity, appearance, duration, and class of the measured antibody.

Methods which use unlabelled materials depend on the expression (or inhibition of the expression) of some measurable function: e.g., neutralization, complement fixation, hemagglutination, or precipitation. The methods are commonly used owing to a large body of experience in their interpretation, the availability of commercial reagents, and the relative simplicity of the required instrumentation. Various techniques are frequently retained not only for convenience, but a difference in the conveyed information: e.g., with rubella, maximal titers typically occur at one to

TABLE 3
Immunodiagnostic Methods

<i>Unlabelled</i>	<i>Labelled</i> (type of label)
Aggregation	Electron dense
—electron-microscopic	Electron spin
—platelet	Enzymatic
Agglutination	Fluorescent
[Direct or Indirect (passive)]	Radioactive
—erythrocytes	
(hemagglutination)	
—other particles	
(latex, bentonite, etc.)	<i>Other</i>
—staphylococcus A	Calorimetric
(coagglutination)	Chemiluminescent
Agglutination-inhibition	Potentiometric
Complement fixation	
(and variants)	
Neutralization	
Precipitation	

two weeks for hemagglutination-inhibition, two to four weeks for complement fixation, but three to seven months for passive hemagglutination [17].

Neutralization can probably be regarded as the standard test. It is useable whenever a virus can be cultivated and its results probably relate more closely to the protective capability of the antibody than the results of any other test. However, it is laborious, expensive, rather insensitive, and its results are typically unavailable for several days or weeks. The neutralizing antibody can combine directly with the receptor, which is responsible for viral attachment to cells, or it can act indirectly. In the latter case, the antibody reacts with a nearby site where it sterically hinders the attachment of the virus. Steric hindrance is increased by a subsequent attachment of a second antibody, directed against the specific antibody, or of the early components of complement (factors C1–C3). Direct damage to the virus occurs with the addition of the later factors (C5–C9). These features can be used to obtain a manyfold increase in the sensitivity of the neutralization test: e.g., by the addition of a heterologous anti-immunoglobulin [24] and complement [23]. Neutralizing antibody is frequently more

TABLE 4
Application of Common Immunodiagnostic Methods

Neutralization	Hemagglutination —Inhibition	Complement Fixation	Immunofluorescence
Adenovirus	Adenovirus	Adenovirus	Adenovirus
Herpesvirus	Coronavirus	Coxsackievirus	Herpesvirus
Lymphocytic choriomeningitis virus (LCMV)	Enterovirus	LCMV	LCMV
Picornavirus	Myxovirus	Myxovirus	Myxovirus
Reovirus	Poxvirus	Poxvirus	Poxvirus
Rhabdovirus	Reovirus	Reovirus	Rhabdovirus
	Togavirus	Rhinovirus	Rubella
		Togavirus	

specific for the strain or type of the infecting virus than antibody assessed by other methods. Neutralization can therefore be used not only to assay the antibody but frequently also to characterize the virus. Modifications of the test have been employed to obtain quicker results or to derive more information about the reactants (for example, the number of neutralized sites on a virus). Such modifications include the plaque reduction assay [25], the use of an immunofluorescent antibody to identify infected cells, and kinetic neutralization assay [26].

The complement fixation assay depends on the addition of a standardized amount of complement (fresh guinea pig serum) to the viral antigen-antibody mixture [27]. The added complement is consumed and therefore is no longer available to lyse an indicator system which consists of sheep red cells bearing a complement-fixing (lytic) antibody (rabbit anti-sheep red cell hemolysin). Fixation of complement occurs chiefly with antibody of the IgM and IgG class (but not subclass 4), which induce the classical pathway of complement activation. Other antibody may activate the alternate pathway. In practice, IgM antibody is sometimes (M. August and A. Baumgarten, unpublished) detected by the test, but not always, owing to its low sensitivity and the small amount of IgM found in circulation (compared to the typically high IgG antibody level).

The assay is widely used but is usually incapable of differentiating between antibody to different strains or types of a virus. Paradoxically, insensitivity is one of the virtues of the assay, because the tested antibody becomes detectable rather late in infection and typically remains so for only a few months. Therefore, antibody associated with current or recent infection is detected rather than antibody which persists after an old infection.

The method is subject to considerable inter- and intra-laboratory variability. The level of antibody also varies considerably between individuals. Assignment of arbitrary titers for "normal" sera is therefore unreliable, though reproducibility of the assay can be maintained over long periods by the use of aliquots of reagents stored below -70°C . An acceptable procedure consists of a simultaneous titration of initial and convalescent sera, the former collected as soon as possible and the latter after an interval of two to four weeks.

The basic assay has been modified in various ways which retain the common feature of utilizing the attachment of complement but assess its effect by different methods and generally with improved sensitivity.

The modifications include:

—Single radial hemolysis [28,29] and single radial complement fixation [30], in both of which the complement exercises its lytic function. The former method uses erythrocytes coated with virus both as the target and the indicator, while the latter method uses virus in the gel as target but a subsequent layer of hemolysin-sensitized red cells in agarose as the indicator.

—Immunoadherence hemagglutination [31], in which bound complement is detected by the adherence of human blood group O erythrocytes in the presence of EDTA to inhibit lysis.

—Assays for the bound factor C3 of complement which is detected by its reaction with labelled anti-factor C3 antibody (by immunofluorescence [32] or EIA).

Hemagglutination and hemagglutination-inhibition can be used to detect either antigen (virus) or antibody. Similar assays, which depend on the aggregation of suitable particles, can be undertaken directly with the virus and the reaction with antibody be observed by electron microscopy [33]. The assays can use other particles and the reaction be detected by various means; e.g., protein A bearing staphylococcus

aureus (which binds the antibody initially) can be observed to undergo coagglutination [34] in the presence of antigen.

In the simplest version, the hemagglutination assay detects the virus by its ability to agglutinate erythrocytes of an appropriate species of animal. Inhibition of the agglutination detects the antibody to the viral receptor for the erythrocyte. In reverse hemagglutination an antibody is used to coat red cells, which are then agglutinated by the virus, a process which can again be inhibited by antibody in a tested fluid. Alternatively, a viral antigen is used to coat the erythrocytes which are then agglutinated by the antibody (passive or indirect hemagglutination). In this case, viral antigen is assessed by its ability to inhibit the agglutination. The hemagglutination reaction for antibody while simple in principle and potentially very sensitive, especially for IgM antibody where its sensitivity can be several thousandfold greater than for IgG antibody, requires a careful (and often elusive) preparation of reagents. The conditions for the preparation of coated red cells include attention to the pH, type, and concentration of buffer, duration of incubation, the temperature, suitable concentration (usually high) of viral antigen (which requires at least partial purification), and an appropriate preparation of the erythrocyte surface. The last may range from simple washing, through the use of tannic acid (at an appropriate concentration) to the addition of stabilizing agents (e.g., formaldehyde) and protein coupling agents [35,36] (gluteraldehyde, chromic chloride, dinitrodifluorobenzene, carbodiimide, etc). The hemagglutination reaction, like the neutralization test, can be used in the kinetic version [37] for improved resolution of antigen.

Precipitation reactions in agarose gels [38] are convenient in that they visibly display each antigen-antibody system which reaches an adequate concentration, but they are usually less sensitive than other methods of assay.

Double immunodiffusion (DI) [38,39] has been used chiefly for the detection of HBs antigen and antibody but also of antibody to CMV, rubella, influenza, adenovirus, vaccinia, rhinovirus, and measles. It is slow, insensitive, and consumes large quantities of material, but is useful in comparing complex mixtures of antigen or antibody.

Counter immunoelectrophoresis (CIE) uses an agarose gel, 0.6–1.0 g/dl, electroendosmotic factor 0.12–0.2, in buffer pH 8.2–8.9. In the gel is cut a 3–5 mm diameter anodal well for antibody and a cathodal well for antigen, 4–6 mm apart. The reactants are driven toward one another by the application of an electric current, 5–10 V per cm, so that unproductive diffusion is restricted. CIE can be several times more sensitive than DI, but is still inefficient for many viruses because of their large size. The disproportion between the migratory speed of the antibody and virus can be reduced by applying the former 10 to 20 minutes before the latter. Obviously, only electronegatively charged antigens can be detected with conventional antisera. The principal advantages of the method are its simplicity and speed (results can be obtained in 30 to 60 minutes). It has been used for the detection of various materials [40,41], including HBs Ag and antibody, rotavirus, enterovirus, as well as antibody to CMV, arbovirus, and myxovirus.

Immunelectrophoresis can be used for the resolution of complex antigenic mixtures, e.g., measles virus [42], but has had only limited application for viral immunodiagnosis. Crossed immunelectrophoresis is a modified method which uses electrophoresis of the antigen in a plain gel in one dimension, followed by electrophoresis into a gel containing antibody in a second dimension, at right angles to the first. Each antigen produces a rocket-like (\wedge shaped) trail of precipitation in which the height of the peak reflects the relative concentration of antigen and antibody.

This method has been used in the study of HSV antigen [43]. An analogous method ("rocket" electrophoresis) uses a single antibody and electrophoresis of the antigen in only one direction, but permits quantification of the antigen by reference to a standard. This method has been used for influenza A [44].

Single radial immunodiffusion (SRID) has been widely applied to the quantification of various protein antigens and can achieve sensitivity as high as $0.165 \mu\text{g/ml}$, but in mixtures with high protein concentration (e.g., serum), a lower sensitivity ($1\text{--}10 \mu\text{g/ml}$) is likely. High sensitivity can be obtained with fixation in $1\text{--}4 \text{ g/dl}$ tannic acid, incorporation of $1\text{--}2 \text{ g/dl}$ polyethylene glycol, MW 4000–6000, in the gel, overlay with a second antibody (immunofixation), or by the use of labelled reagents. However, the method is slow and has limited applicability to whole virus.

Labelled Reagents

Methods which use labelled reagents require only the primary antigen-antibody reaction, but the product has to be separated from unreacted materials before measurement.

Direct methods use either labelled antigen, or antibody, to assay an unlabelled antibody, or antigen, respectively.

Indirect methods use an initial pair of unlabelled reactants, one an antigen, the other an antibody. A second antibody, which is labelled and reacts with the first antibody, is then applied. The amount of the bound second antibody is related to the amount of the bound first antibody which depends, in turn, on the amount of the antigen in the complex.

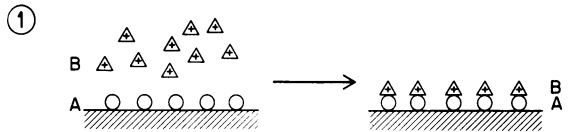
Inhibitory reactions depend on the substitution of all, or a portion, of a labelled material by an unlabelled, but otherwise similar reactant (e.g., an unlabelled antibody which replaces a labelled antibody, with both antibodies having the same specificity for antigen). The reaction takes place in sequence (e.g., antigen, unlabelled antibody, labelled antibody) and the residual amount of bound label is measured.

Competitive assays are similar to the inhibitory ones except that the reactants are mixed, so that the unlabelled antibody competes with the labelled for the antigen in the mixture.

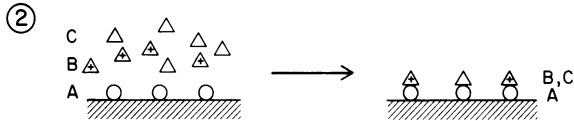
The label can be applied either to the antigen or the antibody, provided that it does not cause a loss of immunoreactivity of the material. The principle is illustrated in Figs. 1–3.

The assays can be used by successive application of the reactants to solid materials (tissue slices, cultured cells, microtiter plates, test tubes, etc.), by mixing with microparticulate materials (cells, plastic spheres, etc.) or in fluid mixtures, after a separation of the bound and residual reactants (e.g., by initial addition of a second antibody and ammonium sulfate, or staphylococcus with protein A [45] and centrifugation, or by gel filtration). The type of label which is used for the assay (Table 3) determines its ultimate sensitivity as well as the required instrumentation.

Immunofluorescence (IF) [46,47] can detect either antigen or antibody by means of an appropriate reactant labelled with a fluorochrome, usually fluorescein, which gives a yellow-green color, or rhodamine, which gives a red color. Reagents can contain both fluorochromes [48], e.g., each fluorochrome on a different antibody, either to characterize distinct antigens (for example, two viruses) or to show that the antigens occur in close association (e.g., on the same virus), when a combined, orange fluorescence is seen. The sensitivity of IF is intermediate between that of unlabelled tests (such as CF), and other methods which use labelled reagents (e.g., EIA or RIA).

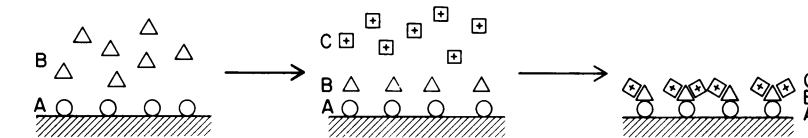


Assay	A	B
Antigen identification	test antigen	labelled antibody
Antibody identification	test antibody	labelled antigen



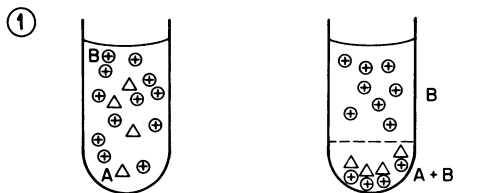
Assay	A	B	C
Antigen quantification	antibody	test antigen	labelled antigen
Antibody quantification	antigen	test antibody	labelled antibody

FIG. 1. Immunoassays using labelled reagents on solid supports (1) Direct assay; (2) Competitive assay.

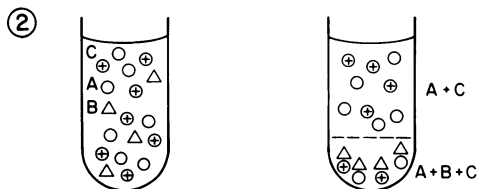


Assay	A	B	C
Antigen identification	test antigen	first antibody	labelled anti-antibody
Antigen quantification	first antibody	test antigen	second antibody, labelled
Antibody identification	antigen	test antibody	labelled anti-antibody
Antibody identification	test antibody	antigen	labelled antibody

FIG. 2. Immunoassay for antigen or antibody on a solid support.



Assay for:	A	B
Antigen	test antigen	labelled antibody
Antibody	test antibody	labelled antigen



Assay for:	A	B	C
Antigen	test antigen	antibody	labelled antigen
Antibody	test antibody	antigen	labelled antibody

FIG. 3. Immunoassay in liquid phase (1) Direct assay; (2) Competitive assay. Initial incubation of the reactants is followed by separation of antigen-antibody complexes before measurement.

More sophisticated techniques, such as fluorescence polarization [49], may further extend the application of the methodology.

Radioimmunoassay (RIA) for protein reactants [50,51] most commonly employs the I^{125} label, which imparts a high sensitivity to the procedure, though others can be used [52]. The iodine can be introduced with chloramine T or by the gentler N-succinimidyl 3-(4-hydroxy 5-iodophenyl) propionate, lactoperoxidase, or electrolytic methods. The labelled reagents become contaminated with degradation products, however, so that the shelf life varies from several weeks to months. A more stable, but much less active, label can be used for protein labelling: e.g., C^{14} or H^3 introduced with dinitrofluorobenzene [53]. The reagents used for radioimmunoassay can also be applied to autoradiography with either conventional or electron-microscopic methods of detection. Another form of RIA, with Cr^{51} , is used for cellular reactants: e.g., in the assay of antibody dependent cellular cytotoxicity [54] in viral infection.

Enzymatic immunoassays [55] use the label either in a manner similar to that for immunofluorescence or radioimmunoassay, as in EIA, or by coupling small antigens (haptens) to the enzyme and measuring the reduced activity of the enzyme which occurs when the active site is blocked by an antibody to the hapten.

EIA-type assays, in turn, can provide continuously variable determination of reactivity, as in conventional, quantitative radioimmunoassay (e.g., for serum protein), or qualitative assay, or step-wise titration, as with immunofluorescence, complement fixation, or neutralization. The assays can use labelled antigen, first or second antibody, or a material which indicates the presence of antibody: e.g., protein A of *Staphylococcus aureus* [56]. The sensitivity of EIA can exceed that of RIA, as the enzyme may be recycled to produce increasing amounts of the measured reaction product. Extreme sensitivity should be obtained with reaction products which are themselves labelled and therefore subject to highly sensitive methods of measurement: e.g., substrates which are, or become, radioactive, fluorescent, photoemissive, or hydrogen donors or acceptors. The last could be measured potentiometrically (e.g., with enzyme-coated electrodes [57]).

Problems in immunodiagnosis result from biological variability of the tested individual, the reagents, and the practitioners of the art, as well as errors in procedure (Table 5). Owing to these variables, "positive" results do not necessarily identify the pathogenic agent nor do "negative" results exclude it. A positive result may be correct in a limited sense, yet be misleading because it fails to detect another, responsible agent: e.g., a virus in multiple infection [58], or an antibody where there is a general increase in immunoglobulin, or an instance of the "original antigenic sin" [59] (i.e., a situation where an antibody to an antigenic determinant, to which the subject is immune, is evoked by another antigenic determinant that is carried by a protein which is similar to the carrier of the original, immunizing determinant).

Monoclonal Antibody

Conventional antisera contain a mixture of a large amount of non-specific immunoglobulins and a small quantity of specific antibodies which differ in avidity and react with various antigenic determinants on the same antigen. Purification of the immunoglobulin fraction (e.g., by ion-exchange chromatography and gel filtration) does not markedly affect the heterogeneity of the antibody, except for restricting the class of immunoglobulin. Affinity chromatography generally results in a loss of the antibody with highest avidity, but, again, does not otherwise alter the heterogeneity. Antibody to contaminants, if it is originally present, also remains in the mixture unless steps are taken to remove it, but such steps often result in a loss

TABLE 5
Causes of Aberrant Results in Immunoassays

Methodological	Biological
Inappropriate method is used	Subject is immunologically unresponsive
Procedure is not observed	Material is inappropriately collected (time, source, procedure)
Incorrect or inactive reactants are employed	Material lacks specificity
Range of effective concentration of reactants is exceeded	Prior vaccination
Inhibitors are present	Multiple infection
Contaminants are present	

of specific antibody, which may be extreme. These difficulties can be overcome with monoclonal antibody.

Monoclonal antibody is produced by a hybridoma [60]. A hybridoma results from the fusion of a non-secreting mouse plasmacytoma cell and a lymphocyte from a suitably immunized subject (mouse, human, etc.). The fusion is effected with polyethylene glycol. Successfully fused cells, selected for their ability to proliferate and secrete an appropriate antibody, are propagated either *in vitro* or in mice.

Monoclonal antibody reacts with only a single antigenic determinant and has uniform avidity. Its ability to recognize an antigen differs from that of conventional antibody to a complex antigen in that only one, not various, antigenic determinants are recognized. Therefore, loss or modification of the determinant results in an inability to recognize an antigen, while an acquisition of the determinant by another antigen is followed by automatic recognition of the second antigen by the antibody. The specificity and uniformity of monoclonal antibody should markedly improve immunodiagnostic accuracy on the one hand [61], while, on the other, it is likely to focus attention on minute differences between similar antigens [62]. The outcome is likely to be the use of a battery of several antibodies to characterize type or group specificity, while the individual antibodies recognize differences between strains. This may have a considerable impact on the understanding of the pathogenic behavior and epidemiology of viral strains.

REFERENCES

1. Ito M, Girvin L, Barron AL: Inactivation of human cytomegalovirus by phytohemagglutinin. *Arch Virol* 57:97-105, 1978
2. Stagno S, Volanakis JE, Reynolds DW, et al: Immune complexes in congenital and natal cytomegalovirus infections of man. *J Clin Invest* 60:838-845, 1977
3. Merigan TC: Host defenses against viral disease. *N Engl J Med* 290:323-330, 1978
4. Smith CB, Bellanti JA, Chanock RM: Immunoglobulins in serum and nasal secretions following infection with type I parainfluenza virus and injection of inactivated vaccines. *J Immunol* 99:133-141, 1967
5. Waner JL, Hopkins DR, Weller TH, et al: Cervical excretion of cytomegalovirus: correlation with secretory and humoral antibody. *J Inf Dis* 136:805-809, 1977
6. Jenner E: An inquiry into the causes and effects of the variolae vaccinae, *Classics of Medicine and Surgery*. Edited by CNB Camac. New York, NY, Dover Publications, 1959
7. Zinkernagel RM, Doherty PC: Immunological surveillance against altered self components by sensitized T lymphocytes in lymphocytic choriomeningitis. *Nature* 251:547-548, 1974
8. Hapel AJ: The protective role of thymus-derived lymphocytes in arbovirus-induced meningoencephalitis. *Scand J Immunol* 4:267-278, 1975
9. Scott R, Kaul A, Scott M, et al: Development of *in vitro* correlates of cell-mediated immunity to respiratory syncytial virus infection in humans. *J Inf Dis* 137:810-817, 1978

10. Buimovici-Klein E, Vesikari T, Santangelo CF, et al: Study of the lymphocyte *in vitro* response to rubella antigen and phytohemagglutinin by a whole blood method. *Arch Virol* 52:323-331, 1976
11. Fryden A, Link H, Moller E: Demonstration of cerebrospinal fluid lymphocytes sensitized against virus antigens in mumps meningitis. *Acta Neurol Scand* 57:396-404, 1978
12. Geme JWS Jr, Yamauchi T, Eisenklam EJ, et al: Immunologic significance of the mumps virus skin test in infants, children and adults. *Am J Epidemiol* 101:253-263, 1975
13. Centifanto YM, Zam ZS, McNeill JI, et al: Leukocyte migration inhibitory factor in HSV infections. *Invest Ophthalm Vis Sci* 17:863-868, 1978
14. Matthews THJ, Lawrence MK: Serum interferon assay as a possible test for virus infection in man. *Arch Virol* 59:35-38, 1979
15. Luster MI, Armen RC, Hallum JV, et al: Immunoglobulin class distribution of measles virus antibodies in serum and spinal fluid of patients with subacute sclerosing panencephalitis. *Int Arch Allergy* 56:488-492, 1978
16. Ogra PL, Morag A, Tiku ML: Humoral immune response to viral infections. In *Viral Immunology and Immunopathology*. Edited by AL Notkins. New York, NY, Academic Press, 1975, pp 57-77
17. Meurman OH: Antibody responses in patients with rubella infection determined by passive hemagglutination, hemagglutination inhibition, complement fixation and solid phase radioimmunoassay tests. *Inf Immun* 19:369-372, 1978
18. Bringuier JP, Andre J, Sohier R: The use of IgM antibody responses in the diagnosis of primary infection to measles, rubella, mumps and M. parainfluenzae viruses. *Med Microbiol Immunol* 164:299-305, 1978
19. Cremer NE, Hoffman M, Lennette EH: Analysis of antibody assay methods and classes of viral antibodies in serodiagnosis of cytomegalovirus infection. *J Clin Microbiol* 8:153-159, 1978
20. Sohier R, de-Thé G: EBNA antibodies are more useful than complement fixing antibodies in monitoring infectious mononucleosis patients. *Biomedicine* 29:170-173, 1978
21. Perelmutter L, Phipps P, Potvin L: Viral infections and IgE levels. *Ann Allergy* 41:158-159, 1978
22. Ljungholm K, Wadsö I, Kjellén L: Microcalorimetric measurement of normal and adenovirus infected HeLa cells. *Acta Path Microbiol Scand B* 86:121-124, 1978
23. Shivers JC, Daniels CA: Enhanced antiglobulin-mediated neutralization of herpes virus-IgG complexes by complement and heterologous anti-immunoglobulin. *Arch Virol* 59:89-98, 1979
24. Sato H, Albrecht P, Hicks JT: Sensitive neutralization test for virus antibody I. Mumps antibody. *Arch Virol* 58:301-311, 1978
25. Lehmann-Grube F, Ambrassat J: A new method to detect lymphocytic choriomeningitis virus-specific antibody in human sera. *J Gen Virol* 37:85-92, 1977
26. Waner JL, Weller TH: Analysis of antigenic diversity among human cytomegaloviruses by kinetic neutralization tests with high-titered rabbit antisera. *Inf Immunity* 21:151-157, 1978
27. Lennette EH, Melnick JL, Magoffin RL: Clinical virology: introduction to methods. In *Manual of Clinical Microbiology*, 2nd edition. Edited by EH Lennette, EH Spaulding, JR Truant. Washington, DC, Amer Soc Microbiol, 1974, pp 667-677
28. Schild GC, Pereira MS, Chakraverty P: Single radial haemolysis: a new method for the assay of antibody to influenza haemagglutinin. *Bull WHO* 52:43-50, 1975
29. Hierholzer JC, Tannock GA: Quantitation of antibody to non-hemagglutinating viruses by single radial hemolysis: serological test for human coronaviruses. *J Clin Microbiol* 5:613-620, 1977
30. Haaheim LR: Single-radial-complement-fixation: a new immunodiffusion technique. Assay of the antibody response to the type-specific antigens of influenza virus in post infection human sera. *Bull WHO* 56:111-116, 1978
31. Lennette ET, Lennette DA: Immune adherence hemagglutination: alternative to complement-fixation serology. *J Clin Microbiol* 7:282-285, 1978
32. Hinuma Y, Ohta R, Miyamoto T, et al: Evaluation of the complement method of fluorescent antibody technique with myxoviruses. *J Immunol* 89:19-26, 1962
33. Vassal JH, Ray CG: Serotyping of adenoviruses using immune electron microscopy. *Appl Microbiol* 28:623-627, 1974
34. Zalan E, Wilson C: Further studies on the Cowan strain of *staphylococcus aureus* as an aid for the diagnosis of influenza. *Arch Virol* 56:177-180, 1978
35. Goding JW: The chromic chloride method of coupling antigens to erythrocytes: definition of some important parameters. *J Immunol Methods* 10:61-66, 1976
36. Habicht GS, Miller F: Preparation of conjugated erythrocytes for long term use in hemolytic plaque assays, complement fixation studies or passive hemagglutinations: a comparative study of several methods. *J Immunol Methods* 11:117-127, 1976

37. Odelola HA, Fabiyi A: Kinetic hemagglutination inhibition technique as a means of detecting antigenic variations among strains of Nigerian flaviviruses. *Arch Virol* 56:291-295, 1978
38. Crowle AJ: Immunodiffusion, 2nd edition. New York, NY, Academic Press, 1973
39. Cappel R, Schluederberg A, Horstmann D: Large-scale production of rubella precipitinogens and their use in the diagnostic laboratory. *J Clin Microbiol* 1:201-205, 1975
40. Middleton PJ, Petric M, Hewitt CM, et al: Counterimmunoelectro-osmophoresis for the detection of infantile gastroenteritis virus (orbi-group) antigen and antibody. *J Clin Pathol* 29:191-197, 1976
41. Gaspar A, Edlinger E: Diagnostic rapide des entérovirus par électro-synérèse. *Ann Microbiol Inst Pasteur* 129:545-552, 1978
42. Vandvik B: Gel-precipitating measles virus antibodies in subacute sclerosing panencephalitis: relation to oligoclonal IgG proteins in the cerebrospinal fluid and serum, and the occurrence of two separate fractions of antibody to virus nucleocapsids. *Acta Path Microbiol Scand C* 85:324-332, 1977
43. Vestergaard BF, Bjerrum OJ, Norrild B, et al: Crossed immunoelectrophoretic studies of the solubility and immunogenicity of herpes simplex virus antigens. *J Virol* 24:82-90, 1977
44. Oxford JS, Schild GC: Quantification of influenza virus structural proteins using rocket immunoelectrophoresis. *J Gen Virol* 38:187-193, 1977
45. Soergel ME, Schaffer FL, Sawyer JC, et al: Assay of antibodies to caliciviruses by radioimmune precipitation using staphylococcal protein A as IgG adsorbent. *Arch Virol* 57:271-282, 1978
46. Swack NS, Michalski FJ, Baumgarten A, et al: Indirect fluorescent-antibody test for human cytomegalovirus infection in the absence of interfering immunoglobulin G receptors. *Infect Immun* 16:522-526, 1977
47. Gardner PS, Grandien M, McQuillin J: Comparison of immunofluorescence and immunoperoxidase methods for viral diagnosis at a distance: a WHO collaborative study. *Bull WHO* 56:105-110, 1978
48. Curtain CC, Baumgarten A: Immunocytochemical localization of the immunoglobulin factors Gm(a), Gm(b) and Inv(a) in human lymphoid tissue. *Immunology* 10:499-506, 1966
49. Levanon A, Inbar M, Kohn A: Fluorescence polarization of DPH-labeled cells adsorbing viruses and its diagnostic potential. *Arch Virol* 59:223-230, 1979
50. Hunter WM: Radioimmunoassay. In *Handbook of Experimental Immunology*, 3rd edition. Edited by DM Weir. Oxford, Blackwell Scientific, 1978, pp 14.1-14.40
51. Rosenthal JD, Hayashi K, Notkins AL: Comparison of direct and indirect solid-phase microradioimmunoassays for the detection of viral antigens and antiviral antibody. *Appl Microbiol* 25:567-573, 1973
52. Baumgarten A, Melrose GJH, Vagg WJ: Preparation of P³²-phosphanilic acid-bovine serum albumin tracer. *Analyt Biochem* 24:243-248, 1968
53. Geczy AF, Baumgarten A: Distribution of a contact sensitizer, 1-fluoro-2,4-dinitrobenzene, in the tissues of the guinea-pig. *Immunology* 22:381-392, 1972
54. Russell AS, Saetre A: Antibodies to herpes simplex virus in "normal" cerebrospinal fluid. *Lancet* 1:64-65, 1976
55. Engvall E: Quantitative enzyme immunoassay (ELISA) in microbiology. *Med Biol* 55:193-200, 1977
56. Madore HP, Baumgarten A: Enzyme-linked protein A: an enzyme-linked immunosorbent assay reagent for detection of human immunoglobulin G and virus-specific antibody. *J Clin Microbiol* 10:529-532, 1979
57. Boitieux J-L, Desmet G, Thomas D: An "antibody electrode", preliminary report on a new approach in enzyme immunoassay. *Clin Chem* 25:318-321, 1979
58. Descamps P, Bedrossian J, Nochy D, et al: Renal transplantation and viral infections. III. Clinical and virological correlations. *Biomedicine* 28:113-119, 1978
59. Francis T Jr: The current status of the control of influenza. *Ann Int Med* 43:534-538, 1955
60. Kohler G, Milstein C: Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur J Immunol* 6:511-519, 1976
61. Wiktor TJ, Koprowski H: Monoclonal antibodies against rabies virus produced by somatic cell hybridization: detection of antigenic variants. *Proc Natl Acad Sci USA* 75:3938-3942, 1978
62. Laver WG, Gerhard W, Webster RG, et al: Antigenic drift in type A influenza virus: peptide mapping and antigenic analysis of A/PR/8/34 (HON 1) variants selected with monoclonal antibodies. *Proc Natl Acad Sci USA* 76:1425-1429, 1979