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Diagnostic Virology: From Animals to Automation

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Methods for diagnosis of viral infection have progressed rapidly during the past two to three decades from animal inoculation to computer automation. Virus isolation, however, still remains the "gold standard." With the availability of antiviral agents, physicians now demand accurate laboratory diagnosis of their patients' illnesses in order to give proper treatment. Discovery of unknown viral agents still requires continued search and diligent effort.

Despite the progress of modern medicine during the past two or three decades, viral diagnostic facilities are still not readily available in most of the hospital-operated microbiology laboratories but generally exist either as part of university research laboratories or regional health departments. Conventional viral diagnostic methods have been time-consuming, expensive, and inaccessible to the practicing physicians; thus an accurate viral diagnosis has infrequently been attempted. In recent years, however, the importance of viral infection has been increasingly recognized, particularly as a cause of serious disease in the immunocompromised patient and in the neonate, as well as a cause of sexually transmitted disease. In addition, new antiviral agents are becoming available. With the advent of effective antiviral therapy, it will no longer be acceptable to hinder or delay the treatment of patients for lack of viral diagnostic facilities. Physicians will undoubtedly demand accurate laboratory diagnosis of their patients' illness in order to institute *specific* and *proper* therapy and management. To accomplish this, viral diagnostic facilities must be more accessible and health practitioners must be more knowledgeable regarding procedures used for viral diagnosis.

VIRAL DIAGNOSIS IN THE EARLY YEARS

In the early years, i.e., prior to 1950, experimental animals were used for virus isolation and diagnosis (Table 1). For example, rhesus monkeys were used exclusively for the isolation of polioviruses [1], whereas newborn mice were essential for the detection of coxsackieviruses [2] and arboviruses [3], or embryonated eggs for influenza viruses (Fig. 1). These techniques were cumbersome, tedious, and time-consuming. Consequently physicians have relied upon alternative methods such as

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TABLE 1
Diagnostic Virology
1925–1985

Year	Method of Detection	Results
1925–1950	Animal inoculation Monkeys Mice Embryonated eggs	Alternative means for virus transmission[a]
1950–1965	Cell culture isolation	Discovery of new viruses
1965–1980	Immunologic methods Radioimmunoassay Enzyme-linked immunosorbent assay Immunoelectron microscopy Many others	Detection of non-cultivable viral agents and/or viral products
1980	Recombinant DNA technology Automation	Detection of viral genome Processing specimens rapidly on a large scale

[a]Prior to 1925, human transmission was necessary to demonstrate causative filterable agents.

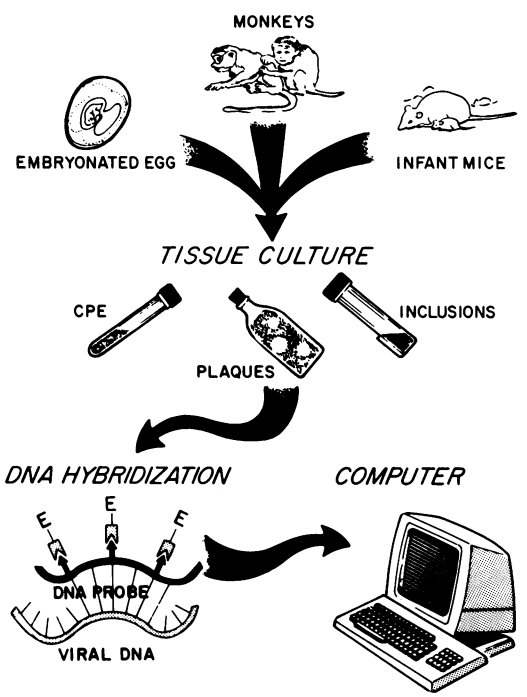

FIG. 1. Diagnostic virology, 1925–1985.

serologic diagnosis using paired serum samples, i.e., one collected during acute illness and one collected during convalescence, from patients suspected of having viral illness. Although retrospective diagnosis offered important information regarding virus activity in the community, its value to patient management was minimal; thus, diagnostic virology gained the reputation that, when the diagnosis was made, the patient had either recovered or expired.

With the discovery by Enders et al. in 1949 [4] that poliovirus can be isolated in cultured cells which were not derived from neural tissue, a new era in diagnostic virology began. The use of cell culture has replaced the use of live animals and provided the measure which diagnostic virologists have used, and are still dependent upon, as the "gold standard" for virus diagnosis. Since then, many new viruses have been isolated and identified as causative agents for many diseases. Furthermore, the cultivation of poliovirus in cell culture not only allowed the diagnosis of infection with this virus but also contributed to the control of this dreadful disease by subsequent development of poliovirus vaccines prepared in cell cultures.

In order to search for a more sensitive cell system for propagation of poliovirus and to provide a large supply of cell cultures for the production of poliovirus vaccines, it was recognized that cell cultures derived from different species of monkeys showed variable degrees of susceptibility to infection with poliovirus and many other related enteroviruses [5], as illustrated in Table 2. Poliovirus types 1-3 and coxsackie B virus types 1-6 induce extensive cytopathic effects (CPE) in both rhesus monkey kidney and patas monkey kidney cell cultures, whereas coxsackie A-9 virus and echovirus types only induce CPE in rhesus monkey kidney cells but not in patas cells. These original observations led to the establishment of selective cell culture systems that are used today for rapid presumptive diagnosis of certain viral infections. Subsequently, Hep-2 cell cultures, a cell line derived from human cancer tissue, were found to have sensitivities similar to the patas monkey kidney cell cultures. More recently, guinea pig embryo fibroblasts were found to be sensitive to certain types of coxsackie A virus, thus eliminating the use of newborn mice for the

TABLE 2
Comparative Sensitivity of Cell Cultures to Enterovirus Infections

	Cell Cultures Derived from			
Virus Types Tested	Rhesus Monkey Kidney	Patas Monkey Kidney	Hep-2 Cell line	Guinea Pig Embryo
Poliovirus types 1-3	+ +	+ + +	+ +	−
Coxsackie B virus types 1-6	+ +	+ + +	+ +	−
Coxsackie A virus type 9	+ +	−	−	−
type 10	−	−	−	+ +
Echovirus types 1-6, 8-14	+ +	−	−	−*

+ + + = highly sensitive
+ + = sensitive
− = resistant
*Echovirus types 7, 8, 9 were tested.

isolation of this group of viruses [6]. With the advent of selective cell cultures and the recognition of distinctive enterovirus plaque morphology [7], diagnostic virology took crucial steps in developing rapid methods for differential identification of viruses. These techniques are similar to those used in a routine bacteriology laboratory for differentiating bacterial isolates, thus establishing simple procedures for presumptive diagnosis of enterovirus virus infections.

DIAGNOSTIC VIROLOGY IN THE MIDDLE 1960s–1970s

In the early 1960s, virology research laboratories in many major medical centers provided limited diagnostic services. Those services were spotty and varied from laboratory to laboratory, depending upon each laboratory's particular research activities. For example, the Yale Poliomyelitis Research Unit offered excellent facilities and trained personnel for the isolation and identificiation of poliovirus and other related enteroviruses; however, methods for detection of other virus groups were not familiar to any of us [8]. It soon became apparent that additional techniques and experience in recognition and characterization of viruses other than the enterovirus group were necessary in order to fulfill the daily demands of a clinical virology laboratory.

In the early 1970s, a new era in diagnostic virology began, because of the development of sophisticated instrumentation and the production of highly purified reagents for detection of many new viral agents. These include radioimmunoassay for the detection of hepatitis B virus antigen in patients' sera [9,10], immunoelectron microscopy for recognition of hepatitis A or B virus particles [11,12] and rotavirus [13,14], and, finally, the development of enzyme-linked immunosorbent assay (ELISA) for the detection of rotavirus antigen in large numbers of stool samples [15]. More recently, the availability and supply of monoclonal antibodies has the potential to make viral diagnosis even more specific and highly sensitive [16]. Thus, virologists are no longer dependent upon laboratory animals and/or tissue culture but may now use alternative immunologic methods for detection of viral antigens (Table 1). More and more, viruses are being discovered as agents associated with many different diseases, and detailed studies of many new viruses have been made possible.

CURRENT STATUS OF DIAGNOSTIC VIROLOGY

With the recent development of new antiviral agents, there has been an increasing demand for definitive diagnosis of infections, particularly those associated with herpesvirus and influenza viruses. It is imperative that the diagnosis be made rapidly and accurately in order to institute effective therapy. How fast can one make an accurate diagnosis of a virus infection? An example is shown in Table 3, for diagnosis of herpes simplex virus infection. The rapidity of the test for the detection of virus in a specimen depends largely upon the number of virus particles present in the original specimen. If there are less than ten infectious virus particles in the specimen, one or two days are necessary when a highly sensitive cell culture system is used. On the other hand, only 30 minutes' examination under the electron microscope is necessary for an answer when 10^6–10^7 virus particles per milliliter are present in the specimen. Such high concentrations of virus in clinical specimens, however, are not commonly encountered. Direct detection of viral antigen in cells by immunofluorescence only requires one to two hours if the number of infected cells in the specimen is sufficient.

Since detection of HSV is the most common request made of the diagnostic

TABLE 3
Examples: Laboratory Diagnosis of Herpes Simplex Virus Infection

Method of Detection	Detection of	Minimum Number of Virus Particles	Time Required for Reporting Results
Cell culture isolation	Viable virus	1–10 infectious virus particles	1–2 days
Electron microscopy	Virus particles	10^6–10^7 virus particles	30 minutes
Immunologic means:			
Immunofluorescence	Viral antigen	5–10 infected cells	1–2 hours
Enzyme-linked immunosorbent assay		10^4–10^6 virus particles	6–24 hours
Nucleic acid hybridization	Viral DNA	10^4–10^6 virus particles	24 hours

virology laboratory today, the method of choice for the detection of HSV in different situations requires a certain degree of consideration. In order to determine the most sensitive cell culture system for the isolation of herpes simplex virus, comparative studies have been made recently by several laboratories [17–20] (Table 4). In primary rabbit kidney cell cultures, 100 infectious virus particles ($TCID_{50}$) will induce CPE in one day, whereas a ten- to hundredfold increase in the number of infectious particles is necessary to induce CPE in human embryo fibroblast (HEF) in two days. In this instance, the Hep-2 cells and Vero cell cultures were less sensitive than

TABLE 4
Comparison of Sensitivities of Different Cell Cultures to Infection with Herpes Simplex Virus

Herpes Simplex Virus Dose ($TCID_{50}$)	CPE Induced by HSV in Different Cell Cultures											
	RK			HEF			Hep-2			Vero		
	1[a]	2	4	1	2	4	1	2	4	1	2	4
10,000	+++[b]	+++	+++	0	+	++	0	+	++	0	++	++
1,000	++	++	+++	0	+	++	0	0	++	0	+	++
100	+	+	+++	0	0	+	0	0	0	0	0	0
10	0	+	++	0	0	0	0	0	0	0	0	0
1	0	0	+	0	0	0	0	0	0	0	0	0

[a] Days post-inoculation
[b] Degree of CPE: +, 25 percent cells showed CPE
++, 50 percent cells showed CPE
+++, 75 percent cells showed CPE

RK = Primary rabbit kidney cells
HEF = Human embryo fibroblast, WI 38/MRC-5; sensitivity of HEF cells varied from cell strain to cell strain.
Hep-2 = Human heteroploid cell line derived from carcinoma of the larynx
Vero = A green monkey kidney cell line
$TCID_{50}$ = Tissue culture infectious dose 50 percent

primary rabbit kidney cells for detection of HSV infections. The additional time necessary to obtain comparable degrees of CPE is important and translates into delayed reporting of results by the clinical laboratory. More recently, conventional tissue culture methods combined with advanced immunologic techniques using avidin- and biotin-labeled reagents have shown the advantage of using such reagents to obtain results more rapidly than by reading CPE alone [21].

FUTURE PERSPECTIVES IN DIAGNOSTIC VIROLOGY

Today the rapid development of numerous immunologic techniques has made significant contributions to viral diagnosis. These techniques have included radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and counterimmunoelectrophesis (CIE) for detecting viral antigens or viral antibodies. In addition, the application of biotin-labeled probes for detection of viral genomes may enhance even more the sensitivity and specificity of viral diagnosis [22,23]. Furthermore, the rapid growth of computer services and automation facilities permit large amounts of information to be fed into machines, allowing better access to and analysis of data obtained in the setting of a diagnostic virology laboratory. It is possible that diagnosis of viral infections in the future will be made by simply pushing a button (Fig. 1).

Exciting progress has been made in the diagnosis of viral infections, progressing from animal inoculation to computer automation during the past half century. However, microorganisms, especially viruses, have their own destiny; as one infection may be controlled, another unpredicted agent may appear. Therefore, regardless of whether one is a basic virologist or a clinical virologist, there is still endless progress to be made in the field of diagnostic virology in the years to come. It will probably still depend upon those with scientific curiosity and diligence, as it has in the past half century.

As a final word, I would like to offer my four "S" criteria for an accurate diagnosis of viral infection and the future of diagnostic virology: *Simplicity, Speed, Sensitivity,* and *Specificity.*

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