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The Impact of Cell Culture Sensitivity on Rapid Viral Diagnosis: A Historical Perspective*

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The contribution of cell culture systems in the diagnosis of viral infections has been well recognized over the years. Not only did such systems make possible the direct isolation and identification of viruses, but also the production of viral diagnostic reagents for rapid diagnosis, the evaluation of antiviral agents, and the production of vaccines for the control of viral diseases. Although many reagents for rapid detection of viral antigens/genomes are currently available, none will make possible discoveries of new viral agents. Thus sensitive cell culture systems are still essential for the rapid and accurate diagnosis of viral infections. Since, as yet, no single cell culture system is susceptible to all viruses, the constant search for additional sensitive cell culture systems for detecting those unknown and/or currently non-cultivable viral agents continues to be an open area of investigation in the field of diagnostic virology.

During the past decade, rapid diagnosis of viral infection has become a reality. There have been significant technological advances; with the commercial availability of specific monoclonal antibodies and viral genome probes, the diagnosis of viral infection has almost reached its golden age. In many clinical situations, however, the viral etiology is uncertain. In order to accomplish a rapid diagnosis in the latter instances, inoculation of clinical specimens into a variety of cell culture systems would provide the best chance of isolating an unknown virus. Moreover, specimens containing very low concentrations of a virus are beyond the limits of sensitivity of current antigen or genome detection methods. Thus cell culture continues to be the "gold standard" of diagnostic virology.

In this paper, the original observations on the differences in sensitivity of cell cultures to viral infection, the benefits accreting over the years from using sensitive cell culture systems for presumptive viral diagnosis, and the current applications of cell cultures for rapid viral diagnosis are briefly reviewed. It is hoped that this review will generate more interest in the search for additional sensitive cell culture systems for the diagnosis of many human diseases of as yet unidentified viral etiology.

Abbreviations: ACV: acyclovir AIDS: acquired immune deficiency syndrome CPE: cytopathic effect GPE: guinea pig embryo HCMV: human cytomegalovirus HEF: human embryonic fibroblast HEK: human embryonic kidney HIV: human immunodeficiency virus HSV: herpes simplex virus MK: monkey kidney ML: mink lung MOI: multiplicity of infection RK: rabbit kidney Vero: African green monkey kidney cell line

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TABLE 1
Original Observations of Differences in Cell Culture Sensitivity to Enterovirus Infections

Enterovirus Types	Cytopathic Effect in Kidney Cell Cultures Derived from	
	Guinea Baboon (*P. papio*)	Yellow Baboon (*P. cynocephalus*)
Poliovirus 1–3	+++[a]	++
Coxsackievirus B 1–5	+++	++
Coxsackievirus A9	++	–
Echovirus 1–14	++	–

[a]Degree of sensitivity:
+++, highly sensitive, extensive CPE
++, moderately sensitive
–, absence of CPE

ORIGINAL RECOGNITION OF CELL CULTURE SENSITIVITY AND ITS IMPORTANCE IN RAPID VIRAL DIAGNOSIS

The differential sensitivity of cell cultures to virus infections has offered a convenient biologic tool for the separation of viruses into subgroups within a group. The original observation that poliovirus can be distinguished from Coxsackieviruses and echoviruses by their ability to replicate in cell cultures, derived from different monkey species, was an incidental finding [1]. In order to search for a more sensitive cell culture system other than rhesus monkey kidney cells for propagating poliovirus, kidney cell cultures prepared from two species of baboons were tested for their sensitivity to poliovirus and other enterovirus infection. Surprisingly, kidney cell cultures from the two species of baboons showed different degrees of sensitivity to poliovirus and Coxsackievirus group B and those of Coxsackievirus group A and echovirus types (Table 1). Cell cultures derived from guinea baboon (*Papio papio*) were sensitive to all enteroviruses tested at that time, but cell cultures derived from yellow baboon (*Papio cynocephalus*) were completely resistant to infections with Coxsackievirus type A9 and all 14 types of echoviruses. Since baboon cells were not readily available, other cell lines, including Hep-2 cells and patas monkey kidney cells, were tested and found to have different degrees of sensitivity to enterovirus infections (Table 2). These cell culture systems have subsequently been used as markers for identifying the enteroviruses [2,3]. These original observations led to the establishment of sensitive cell culture systems that are used today for rapid presumptive diagnosis of viral infections [3,4,5].

ILLUSTRATION OF SENSITIVE CELL CULTURE FOR RAPID PRESUMPTIVE DIAGNOSIS OF AN ECHOVIRUS INFECTION

The first case of a rapid presumptive diagnosis of an echovirus infection by cell culture sensitivity was made in 24 hours from a patient with Bornholm disease [6]. Although the patient showed classical symptoms of pleurodynia with which Coxsackievirus group B was usually associated, the results obtained from the patient's throat swab specimen inoculated into two types of monkey kidney cells indicated that an echovirus had been isolated. Since the isolate only induced cytopathic effect (CPE) in rhesus monkey kidney cells but not in patas monkey kidney cells (Fig. 1), therefore,

TABLE 2
Comparative Sensitivity of Cell Cultures Derived from Human, Simian, and Non-Primates to Human Enterovirus Infections

	Cytopathic Effect in Cell Cultures[a] Derived from					
	Human Cell Line		Simian Cells		Non-Primate Cells	
Enterovirus Type	HEF	Hep-2	Rhesus	Patas	RK	GPE
Poliovirus 1–3	++[b]	++	++	+++	–	–
Coxsackievirus B 1–6	++	++	++	+++	–	–
Coxsackie A9	+	–	++	–	–	–
Coxsackievirus A 2–6,8,10,12	–	–	–	–	–	++
Echovirus 1–14	+	–	++	–	–	–

[a]Cell cultures:
HEF: human embryo fibroblast cells
Hep-2: human epidermoid carcinoma cell line
Rhesus: rhesus monkey kidney cells
Patas: patas monkey kidney cells
RK: rabbit kidney cells
GPE: guinea pig embryo fibroblast cells
[b]Degree of sensitivity:
+++, highly sensitive, extensive CPE
++, moderately sensitive
+, fairly sensitive
–, absence of CPE

according to Table 2, the isolate would either be Coxsackievirus A9 or an echovirus, but not Coxsackievirus group B. Subsequently, the isolate was identified as echovirus type 8, which was confirmed by an antibody rise in the patient's serum against the echovirus type isolated but not to any type of the Coxsackievirus group B [6]. Had the two cell culture systems not been used, a 24-hour presumptive diagnosis of an echovirus infection would not have been possible. Moreover, had only the Coxsackievirus group B infection been sought, it would have resulted in a negative report. A diagnosis of echovirus infection would have been completely missed.

INCIDENTAL FINDING OF A NON-PRIMATE CELL CULTURE SYSTEM SENSITIVE TO HUMAN ENTEROVIRUS INFECTION

It is often an incidental finding that a new cell culture system is discovered to be sensitive to the infection of a specific virus. This type of event was how the ability of Coxsackievirus group A to replicate in primary guinea pig embryo (GPE) fibroblast cell cultures was detected [7]. This group of viruses generally propagates well only in suckling mice; however, when a throat swab obtained from a child with herpangina was inoculated into several cell culture systems, including human embryonic kidney, rhesus monkey kidney, and GPE cells, rapid virus-induced CPE was observed only in GPE cells. The isolate was identified as Coxsackievirus A10. Subsequently, several other Coxsackieviruses group A, including A2–6, 8, and 12 were also found to replicate in GPE cells [7]. Had the GPE cells not been used in this case, the usual procedure for isolation and identification of a Coxsackievirus group A by inoculation of infant mice would have taken several weeks. Although GPE cells are seldom used in a routine

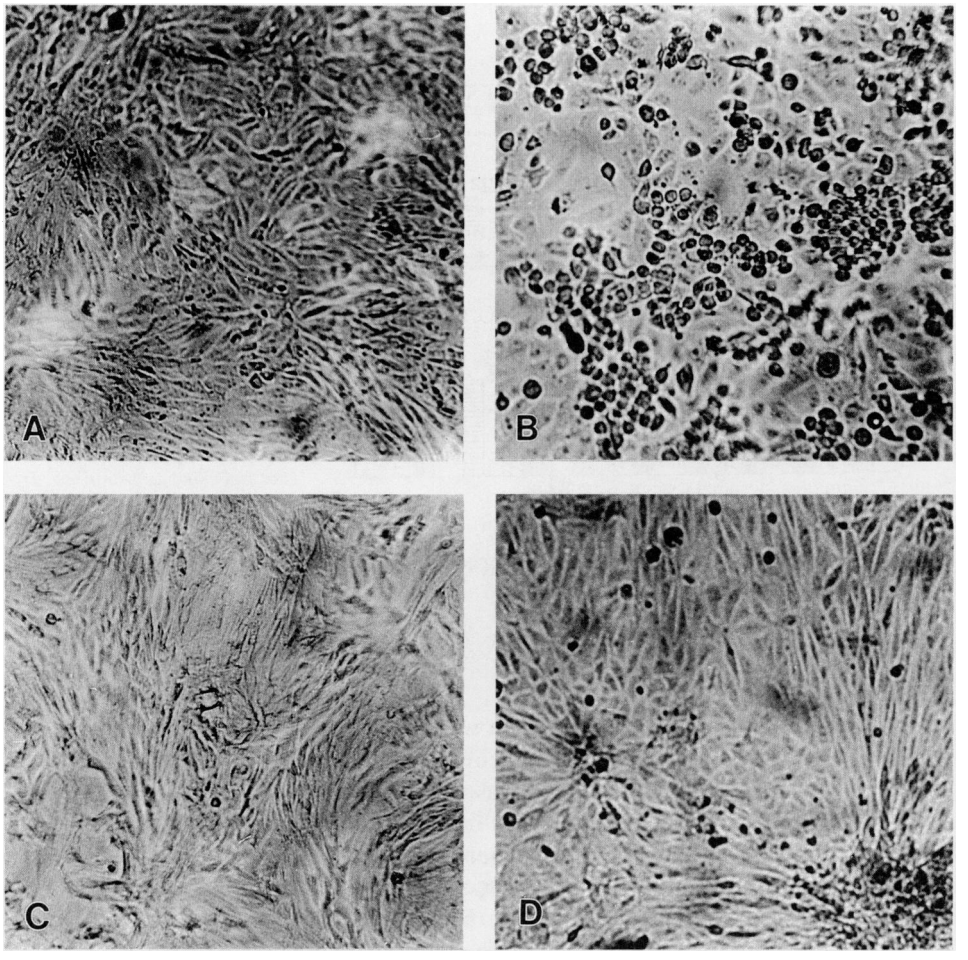

FIG. 1. Cytopathic effect induced by an echovirus in rhesus monkey kidney but not in patas monkey kidney cell cultures. **A.** Uninfected rhesus monkey kidney cell culture. **B.** Rhesus monkey kidney cell culture infected with an echovirus showing CPE 24 hours post-inoculation. **C.** Uninfected patas monkey kidney cell culture. **D.** Patas monkey kidney cell culture infected with an echovirus; note absence of CPE seven days post-inoculation.

clinical laboratory, this case merely demonstrates that a rapid diagnosis of viral infection can be made when a sensitive cell culture system is used.

ADVANTAGE OF SENSITIVE CELL CULTURE FOR RAPID DIAGNOSIS OF HERPES SIMPLEX VIRUS OR ADENOVIRUS INFECTION

Herpes simplex virus (HSV) is the most common virus type encountered in a clinical virology laboratory today. If an insensitive cell culture system is used for its detection, however, a delayed or even a negative result may be obtained. In general, primary rabbit kidney (RK) cell cultures are more sensitive to HSV than Vero cell cultures, a cell line derived from African green monkey kidney commonly used in many laboratories [8]. This fact was also illustrated in clinical situations (Fig. 2). When clinical specimens contained <50 HSV particles/0.1 ml there was no virus-induced

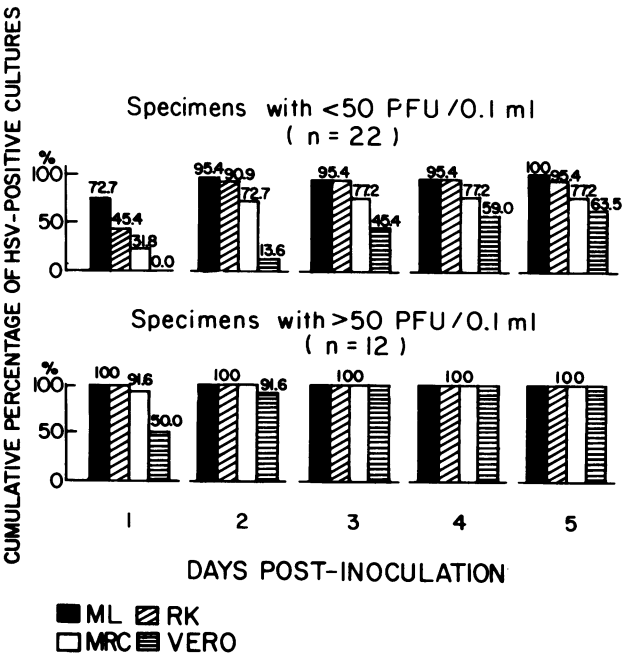

FIG. 2. Cumulative percentage of HSV-positive cultures of clinical specimens, showing cytopathic effect induced by HSV in four different cell culture systems by days post-inoculation. Specimens containing <50 PFU/0.1 ml (*top line*) and >50 PFU/0.1 ml (*bottom line*). Modified from [10]. **ML:** mink lung cell line **RK:** rabbit kidney cells **MRC:** human embryo fibroblastic cells **VERO:** African green monkey kidney cell line.

CPE in the Vero cell, although 73 percent were virus-positive in a sensitive mink lung (ML) cell line and 45 percent in RK cell culture in one day [9,10]. On the other hand, when the specimens contained >50 virus particles/0.1 ml, there were 50 percent isolations of HSV in Vero cells in one day but 100 percent in ML and RK cells. Thus, rapid viral diagnosis can be made only when the *proper* sensitive cell culture system is used. Rapid detection of HSV in clinical specimens with ML and/or RK cell cultures has been reported subsequently by many other laboratories [11–14].

Similar benefit has been obtained with the isolation of adenovirus when a proper cell culture system is used [15]. As shown in Table 3, an early diagnosis of an adenovirus infection can be facilitated when human embryonic kidney (HEK) cell cultures are

TABLE 3
Comparison of Three Cell Culture Types for Recovery of Adenovirus from Clinical Specimens[a]

Clinical Specimen	Total No. Tested	Cell Culture (%)				
		Sensitivity			Detected Earlier in	
		HEK[b]	HEF	MK	HEK	HEF/MK
Eye swab	60	97	79	ND[c]	54	0
Respiratory tract secretion	89	100	73	64	38	2

[a]Modified from [15]
[b]HEK: human embryonic kidney
HEF: human embryonic fibroblast
MK: primary monkey kidney
[c]ND: not done

used. If, however, only monkey kidney cell cultures are used, a delay in diagnosis is likely to result and, in certain situations, one may not even isolate the adenovirus. Unfortunately, we do not have a single cell culture system which is sensitive to the infection of all viruses; therefore, we have to select the proper cell culture systems or use several cell culture systems for virus isolation, especially in cases when we do not have a specific virus in mind.

EARLY DETECTION AND IDENTIFICATION OF VIRUS INFECTION AFTER CELL CULTURE AMPLIFICATION

Immunologic and hybridization techniques can be applied to virus-infected cell cultures for a more rapid and accurate diagnosis of viral infection. This fact is of particular importance for slow-growing viruses, such as human cytomegalovirus (HCMV). In general, the isolation of HCMV requires seven to ten days. The recent application of the centrifugation of clinical specimens on to cell cultures in shell vials followed by detection of HCMV early antigen by immunologic staining techniques made possible the diagnosis in only 16–24 hours post-inoculation [16–18]. Centrifugation of specimens on to shell vial cultures to amplify virus infection, followed by the use of monoclonal antibodies for viral identification, is a great advantage in rapid viral diagnosis, not only for HCMV, but also for the detection of other virus infections, including influenza virus and adenovirus [19,20].

Nucleic acid hybridization has also been applied to the detection of virus genome in clinical specimens. When dot-blot hybridization was used for the detection of HCMV in urine samples, the time required for the detection was greatly shortened; however, 10^3 to 10^5 virus particles per milliliter of urine were necessary for a positive result [21]. Subsequently, the development of in situ hybridization for the detection and localization of virus genome in infected cells and/or tissues was another advance in technology [22]. When, however, the immunologic technique for detecting viral antigen was compared with the hybridization technique for detecting viral genome of HSV-infected GPE or HEF cells, it was found that the cell culture systems used for the initial amplification of HSV were important [23]. When a high dosage of virus inoculum was used, i.e., >1.0 multiplicity of infection (MOI), viral antigen and viral DNA could both be detected as early as four to eight hours post-infection in both cell culture systems, earlier than virus-induced CPE in either cell culture system. On the other hand, when the input MOI was $<.0001$, positive results were not obtained by either technique until 12–16 hours post-inoculation, at which time CPE was already noticeable in the sensitive GPE cell culture system. Thus, virus-induced CPE alone in the sensitive GPE cell culture system was as rapid as viral antigen or viral DNA detection in the less sensitive human embryonic fibroblast (HEF) cell culture system [23].

KNOWLEDGE OF CELL CULTURE SENSITIVITY FOR EVALUATION OF ANTIVIRAL AGENTS

Today, chemotherapy for viral infection has become increasingly available. Once a virus is isolated and identified, it will soon be necessary to know which of a variety of antivirals is most appropriate for treatment of the patient, a situation similar to antibiotic testing for bacterial isolates. Some antivirals are toxic; therefore, it is important to use them only when absolutely necessary. The basic procedure for antiviral testing lies in the inhibition of virus replication, which in turn requires growth

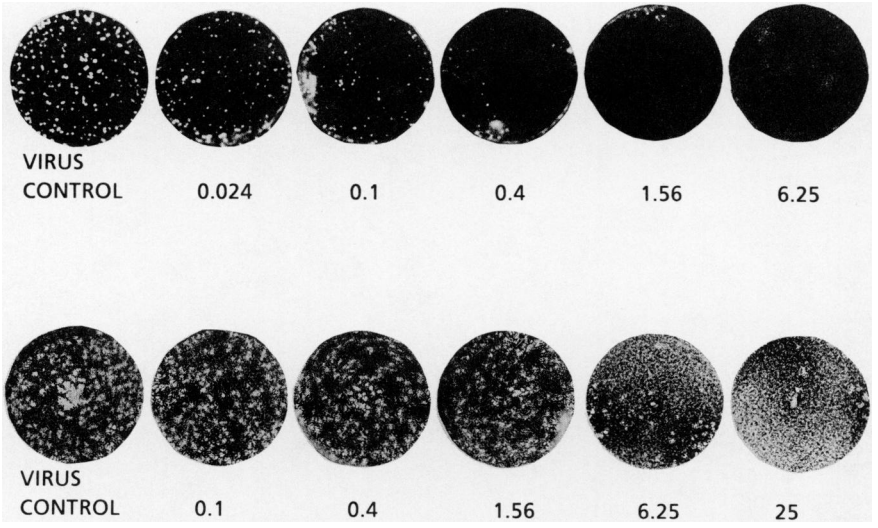

FIG. 3. Comparison of antiviral effect of ACV against HSV-1 infections in two different cell culture systems; Vero vs. GPE: Top row, a 50 percent reduction of plaque formation was obtained at 0.4 μM of ACV in Vero cells, whereas as shown in bottom row, 6.25 μM of ACV was necessary in GPE cells.

in cell culture. Once again, when different cell culture systems are used, different results may be obtained. As illustrated in Fig. 3, when highly sensitive cells, for example GPE cells, are used to assay acyclovir (ACV) inhibition of HSV-1 replication, a higher concentration of drug is needed; whereas a lower concentration of drug is required when Vero cells are used for the assay [24]. Thus, the importance of selecting a proper cell culture system for drug sensitivity testing is readily apparent, but the mechanism for these results is still not clear.

CHANGING INTEREST IN VIRUS GROUPS IS INFLUENCED BY ADVANCES IN TECHNOLOGY, BUT ITS ROOTS LIE IN CELL CULTURE

Currently, there are many illnesses suspected of being caused by virus infection without any definite etiologic agent. The use of cell culture would provide an open-minded search for an unknown agent. Once the viral agent is isolated and identified, means for the control of the disease can be developed. This hypothesis is exemplified by the isolation and cultivation of poliovirus in cell culture [25] followed by numerous studies, as reviewed by Paul [26], which led to the production of poliovirus vaccines, and ultimately to the control of this dreadful viral disease in the early 1960s. Thus, our interest in studying the poliovirus group has gradually declined from 1960–1980 [27].

Although recent developments allowing detection of viral antigen and/or viral genome directly in clinical specimens provide more rapid viral diagnosis, in many instances these techniques are not as sensitive as cell culture isolation, due to low concentration of virus in clinical specimens. Furthermore, both antigen and genome detection are directed only to the suspected virus; thus, an unexpected or a new virus would not be detected, as exemplified earlier in the isolation of echovirus type 8 from a

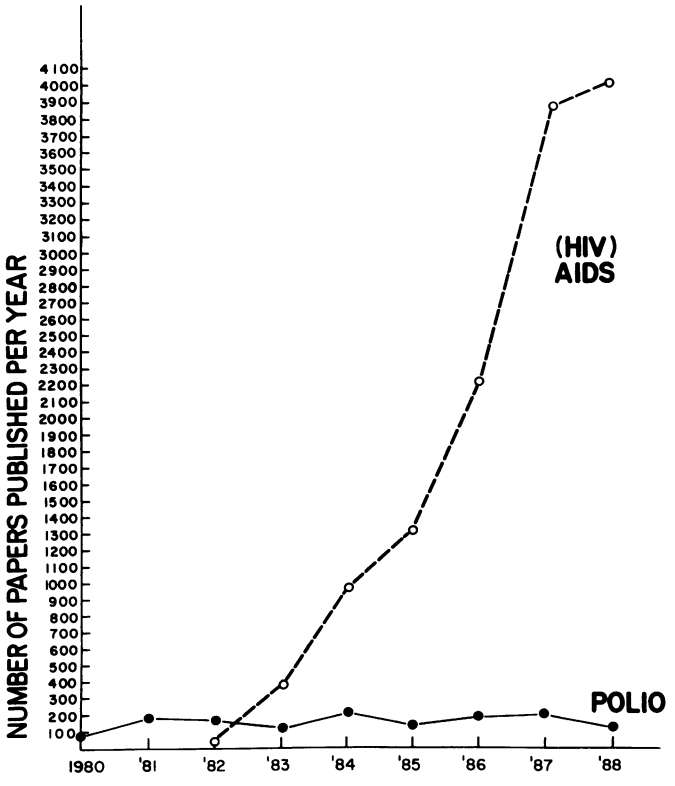

FIG. 4. Number of publications recorded in the *Index Medicus*, 1980–1988, on poliovirus/poliomyelitis vs. HIV/AIDS.

case of pleurodynia [6]. Similarly, the tremendous progress in the investigation of the epidemic of human immunodeficiency virus (HIV) infection was not possible until success in the isolation and propagation of this virus in cell cultures was attained in the early 1980s [28–32]. The establishment of a cell line, H9, from a leukemic patient facilitated the propagation of the virus and the production of large quantities of HIV antigen [29]. Large-scale production of the virus permitted the development of serologic tests for screening blood samples and epidemiologic surveys, the production of monoclonal antibody for viral antigen detection and various immunocytochemistry staining, and the preparation of cloned viral genome probes for studies of the molecular mechanisms of HIV infection. As a result, a vast number of published papers may be found in the *Index Medicus* (Fig. 4); however, their roots have been derived from cell culture isolation. Unfortunately, we have yet to discover a more convenient and sensitive cell culture system for the isolation of the HIV group of viruses, and, more important, to develop an effective vaccine or antiviral agent for the control of this debilitating and life-threatening viral disease.

CONCLUDING REMARKS

The use of cell culture over the past 30 years has provided us with a most convenient biologic method for the isolation and identification of a variety of viruses and control of many viral diseases. There are still hundreds of cell lines derived from human and non-human tissues that, as yet, are unstudied for human viral infection. Since there is not a single cell culture system equivalent to the blood agar plate used in a bacteriology

laboratory, one which can be used for the isolation of all or even most viruses, a continued search for the most sensitive cell culture system to infection by a given virus remains an open area of investigation in the field of diagnostic virology.

This review has only briefly discussed the original observations on the differences in sensitivity of cell cultures to viral infection and the advantage of using sensitive cell cultures for the isolation and identification of viruses for rapid viral diagnosis. It is hoped that this review will generate more interest in research in clinical virology during this golden age of molecular medicine in order to combat some of the devasting human viral diseases.

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