Electron Microscopy for the Rapid Detection and Identification of Viruses from Clinical Specimens

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The advantages of using electron microscopy for rapid diagnosis of virus infection from clinical specimens, for identification of virus isolates with unusual properties, and for monitoring endogenous agents in cell cultures are illustrated by several actual cases that have occurred over the years. The importance of using morphological characteristics of viruses for initial identification is emphasized.

INTRODUCTION

With the advent of antiviral chemotherapy, the demand for timely and specific diagnosis of viral infection has become considerably greater than it was a decade ago. Conventional methods for the definitive diagnosis of viral infections consist of culture isolation and serologic confirmation of the etiologic agents. Alternatively, determination of antibody rise in patient sera taken during the acute and convalescent stages of infection may help in the diagnosis of virus infection, although the information may be too late for patient management.

The application of electron microscopy (EM) in the laboratory diagnosis of virus infection offers some advantages which can be utilized favorably for a rapid and accurate diagnosis in various situations. EM can identify both infectious and noninfec-
tious virus particles as well as those viruses that are unable to replicate in conventional cell cultures. EM identification of virus is based on virus morphology and the ultrastructure of virus-cell interaction; it is not restricted by the narrow specificity of viral test reagents used in most rapid immunodiagnostic methods. Furthermore, conventional methods for identification of virus isolates often rely on their biological and antigenic properties. In some instances, a virus isolate does not possess classical characteristics useful for identification but instead has unusual properties, which make identification more difficult when conventional methods are used. In such situations, the use of electron microscopy has provided an alternative approach to rapid and accurate diagnosis.

The following brief review is focused on some practical applications in a clinical

Abbreviations: AIDS: acquired immune deficiency syndrome CPE: cytopathic effect CMV: cytomegalovirus DNA: deoxyribonucleic acid ELISA: enzyme-linked immunosorbent assay EM: electron microscopy HIV: human immunodeficiency virus HPV: human papilloma virus HSV: herpes simplex virus IEM: immune electron microscopy IFA: immunofluorescence assay MCV: molluscum contagiosum virus NS: negative staining PTA: phosphotungstic acid RNA: ribonucleic acid SV-5: simian virus type 5 SV-40: simian virus type 40 VZV: varicella-zoster virus

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laboratory of EM as an approach to rapid, accurate laboratory diagnosis of virus infection. The purpose of this discussion is not to present an extensive review of the literature. Rather, it is intended to concentrate on the unique contribution of EM in its practical application to clinical virology, especially with those viruses possessing unusual features. Therefore, the selection of references is confined to either original discoveries or to review papers.

EM TECHNIQUES FOR SPECIMEN PREPARATION

Two major techniques commonly used in the electron microscopy laboratory for virus study are negative staining and thin sectioning. Detailed descriptions of these procedures can be found in several review papers [1–5].

Negative Staining

Negative staining (NS) has been one of the most useful techniques for visualization of virus particles since its first application in virology by Brenner and Horne in 1959 [6]. It is the method of choice for rapid identification of virus particles in clinical specimens because of its simplicity, rapidity, and high resolution. A suspension of the clinical specimen is deposited on to a Formvar membrane-coated specimen grid and then stained with a 1–2 percent aqueous heavy metal salt solution, such as sodium or potassium salt of phosphotungstic acid (PTA), at pH 6–7. Afterward the specimen is dried and irradiated by ultraviolet light to inactivate the virus. The grid is then ready to be examined. Fluid specimens containing a high titer of virus particles, such as vesicle fluid, stool extract, and serum, are suitable for direct examination using the NS technique. The entire procedure requires approximately 30 minutes. The limitation of this technique is the need for a large number of virus particles (≥10^6/ml) in the specimen in order to detect the virus. Various methods have been developed to increase virus concentration and hence the sensitivity of the technique.

Methods to Enhance Virus Visualization

Methods routinely used for enhancing virus visualization in clinical specimens are:

Ultracentrifugation: Ultracentrifugation is commonly used to spin down virus particles. A viral specimen should first be subjected to a low-speed centrifugation to remove gross debris. The clarified supernatant is then centrifuged at a higher speed (15,000 g) for one hour, as recommended by Almeida [7]. Pellets are resuspended in distilled water, which helps to lyse cellular structures but leaves the virus intact. More recently, a table model Airfuge (air pressure-driven ultracentrifuge) has become available for spinning minute amounts of virus suspension directly on to EM grids [8]; this procedure could increase virus concentration a hundredfold. After centrifugation, the grids can be stained with PTA and examined.

Agar-gel diffusion: This method was first introduced by Kellenberger and Arber in 1957 [9] and applied to concentrated virus for routine EM examination by Anderson and Doane [10]. It is a simple method and easy to use. A drop of viral suspension is placed on a piece of agar block (1–2 percent) on a microscope slide. A Formvar carbon-coated specimen grid is allowed to float upside down on the virus suspension. Water, salts, and proteins of low molecular weights diffuse through the block agar, leaving the virus particles concentrated in the droplet on top of the agar block to adhere to the grid.

Immune electron microscopy (IEM): Immune electron microscopy has been widely used for rapid immunodiagnosis of virus infection. It is also a method for enhancing the
visualization of virus particles for electron microscopic examination [11]. Immune serum containing specific antibodies to the particular virus suspected is mixed with the specimen, followed by one hour of incubation at room temperature in order to allow antigen-antibody reaction to take place. Virus particles form aggregates by reaction with their homologous antibody. Small virus particles such as Norwalk virus [12] and hepatitis A virus [13] from feces often require use of this technique in order to facilitate the detection and differentiation of the virus particles from background materials. The virus-antiserum mixture can be further concentrated by agar-gel diffusion or ultracentrifugation, as described above. Alternatively, the antiserum can be first absorbed to the grid as a solid phase, after which the virus is applied to the grid and then permitted to form antigen-antibody complexes for visualization [14].

For the purpose of immunodiagnosis, following primary antigen-antibody reaction, ferritin or colloidal gold-labeled secondary antibody (antibody against primary antibody) can be applied to the mixture [15,16]. The antigen-antibody complexes are then made visible in the electron microscope. This procedure is useful for identifying viruses of different antigenic types within the same virus group.

Thin Sectioning

Although the method is less rapid, examination of thin sections of virus-infected cells, which have been properly fixed and embedded, provides a more reliable diagnosis, especially when the virus structure is not distinct using routine NS technique. Thin-sectioning technique has the advantage of allowing the observation of virus-cell interaction, which reveals the site of virus replication and maturation in the host cells, information pertinent to the identification of unknown viruses. On the other hand, the disadvantages of the thin-sectioning method are technical: more time for specimen preparation is needed, as are trained personnel with special skills.

The conventional procedures consist of primary fixation with glutaraldehyde or paraformaldehyde (2–4 percent), post-fixation with osmic acid (1–2 percent), en bloc staining with uranyl acetate to enhance contrast (an optional step), dehydration with ethanol, infiltration with propylene oxide, embedding in epoxy resin or some other embedding media, thin sectioning, and staining. Detailed procedures can be found in many electron microscopy procedure writings, such as those cited in the references [4,17–19]. In large medical centers today, EM is standard equipment in most pathology laboratories, and personnel with special skills are available to assist in specimen preparation. Therefore, the availability of EM diagnosis for virus infections should not be a major problem.

APPLICATION OF EM IN CLINICAL VIROLOGY

Diagnosis of Poxvirus and Herpesviruses from Vesicle Fluid

The advantage of using EM for the identification of unknown viruses from clinical specimens has long been recognized. Historically, EM has been used successfully to detect virus in vesicular fluid or pustular material for the diagnosis of smallpox and varicella-zoster virus (VZV) [20–21]. EM provides a method for rapid, accurate laboratory diagnosis of virus infection and thus for proper patient management. Although smallpox has been eradicated, other poxviruses, such as vaccinia virus, can cause generalized infection in immunocompromised hosts and require immediate diagnosis. The prevalence of herpes simplex virus (HSV) infection has been increasing in recent years; EM can provide rapid diagnosis of it. Morphologically, poxviruses are
large, DNA-containing viruses, exhibiting a brick shape, $300 \times 200 \times 100$ nm in dimension, and with a complex surface structure (Fig. 1A). The herpesviruses commonly causing skin lesions are HSV or VZV. All herpesviruses have the same morphology; they are spherical enveloped viruses with a diameter of 150 nm. Inside each envelope, a unique icosahedral-shaped nucleocapsid, consisting of deoxyribonucleic acid (DNA) and surrounded by 162 capsomers of protein subunits, is easily identified (Figs. 1B, 1C).

Detection of Virus Particles in Stool Specimens from Patients with Nonbacterial Gastroenteritis

In the early 1970s, several groups of investigators simultaneously discovered, by electron microscopic examination, a new virus agent named "rotavirus," which was responsible for many cases of infantile gastroenteritis [22,23]. EM was the only method for rotavirus detection during those years. Morphologically, rotavirus particles are spherical, 70 nm in diameter, consisting of ribonucleic acid (RNA) surrounded by a double layer of capsomers and exhibiting a wheel-like appearance (Fig. 2A).

Subsequently, enzyme-linked immunosorbent assay (ELISA) kits became available for rotavirus detection [24,25]. ELISA only detects rotavirus which shares a common antigen to the virus used in the preparation of the antiserum furnished in the kit; any new viral strain that lacks the common antigen will not be detected by this reagent.

In 1984, we had an unusual experience while examining the stool specimen of a patient from China with epidemic diarrhea: EM examination revealed virus particles with rotavirus morphology, but the specimen was negative by ELISA (Abbott Laboratories). Later in that year, Hung et al. reported a virus, isolated from stool
samples of adult diarrhea patients from China, that resembled rotavirus in morphology, but which, antigenically, lacked the group antigen shared by known rotaviruses [26]. Using ELISA only, without EM examination, our result would have been a false negative. This incident illustrates the advantage of using EM to discover new viruses or viruses known but antigenically different from the prototype strain.

In addition to rotavirus, other virions such as adenoviruses, astrovirus, calicivirus, coronavirus, and enteroviruses are often detected in stool specimens of patients with nonbacterial diarrhea or other gastrointestinal disorders [27-30].

Enteric adenoviruses (types 40 and 41), often associated with infantile gastroenteritis, are 70–80 nm in diameter, with distinct icosahedral symmetry [27-29]. Enteric adenoviruses are often present in stools in large numbers and can be detected by direct electron microscopic examination without difficulty (Fig. 2B). These adenoviruses are difficult to grow in conventional cell cultures. Until very recently, while other immunologic methods of detection were being developed, EM was the only method for detecting these agents in stool specimens.

Norwalk agent, a small, picorna-like virus, 27 nm in diameter, is responsible for outbreaks of gastroenteritis among adults and school children during the winter months [12,30]. Outbreaks have occurred in recreational camps, on cruise ships, in schools, and in nursing homes as a result of drinking or swimming in contaminated water or eating uncooked shellfish [31–33]. Identification of Norwalk virus is done initially by immune electron microscopy (IEM), using convalescent serum containing antibody to Norwalk virus [12].

**Virus in Urine Specimens**

Cytomegalovirus (CMV) has been frequently isolated from urine specimens of children with congenital infection [34], of immunocompromised patients (including organ transplant recipients), and of patients with acquired immunodeficiency syndrome (AIDS). Isolation of the virus in cell cultures requires from several days to weeks before signs of virus activity become evident. CMV can be directly observed by EM examination, using NS [35,36] or thin sectioning [37]. CMV particles in urine specimens are usually in low concentration; therefore, it is necessary to concentrate the urine specimens by ultracentrifugation. As a supplemental method to tissue-culture isolation, EM can provide a rapid diagnosis of CMV infection. Now CMV early
antigen can be rapidly detected (within 16 hours after incubation of the centrifugation culture) [38]; this technique has an advantage over EM because it is easily adapted in smaller clinical laboratories that lack an EM.

One limitation of the technique is that, in addition to CMV, HSV has occasionally been isolated from the urine of immunocompromised patients. EM examination of NS preparations alone cannot differentiate CMV from HSV because the viruses have the same morphology; observation of thin sections of virus-infected cells for characteristic cytopathology (Fig. 3A) or the use of IEM may aid in identifying CMV or HSV.

Papovavirus is another virus often present but unnoticed in urine specimens of immunosuppressed patients [39,40]. This group of viruses is not usually isolated by conventional methods because of a lack of distinct cytopathic effect (CPE) in cell cultures and a slow replication, requiring two to three weeks to produce CPE. EM can provide a definitive identification, however, because papovavirus has a distinct morphology and can be recognized without difficulty. In papovavirus-infected tissue-culture cells, identification can be achieved by negative staining of the supernatant of infected culture fluid or by thin sectioning of virus-infected cells. Based on the size, shape, and location of virus particles in the cells, one can make a presumptive diagnosis (Fig. 3B).

Our experience indicates that EM is the method of choice for papovavirus identification. In 1981, an unidentified virus isolate was sent to us for EM identification from the Virus Laboratory of Massachusetts General Hospital. This isolate came from a urine specimen of a renal transplant recipient. The virus produced CPE in human embryonic lung and monkey kidney cells, with low virus infectivity titer; the CPE was not characteristic of any of the known common human viruses. The virus was resistant to chloroform treatment, indicating that it was devoid of an envelope. Upon EM examination, this isolate was identified immediately as papovavirus, based on virus morphology.

**Direct Examination of Biopsy and Autopsy Tissues**

When a virus infection is suspected, a specimen of biopsy or autopsy material can be obtained, fixed, and processed for electron microscopy.

**Observation of Virus in Brain Tissues**

The availability of chemotherapy for herpessvirus encephalitis prompted the need for a definitive diagnosis of HSV infection, a case of which is illustrated by a thin section of brain tissue in Fig. 4A. Although tissue-culture isolation of HSV remains the most sensitive method for its detection, positive EM identification provides direct proof of virus presence in the specimen and rules out any possibility of laboratory contamination.

Other viruses have been observed in brain tissues, such as papovavirus associated with progressive multifocal leukoencephalopathy [41]. EM remains the method of choice for detection since the isolation of papovavirus in cell culture by conventional methods is difficult.

Human immunodeficiency virus (HIV) can cause neurological disorders in many AIDS patients. HIV particles have been found in their brain tissues [42]. Isolation of the virus in cell culture or the detection of viral antigen in brain tissues may be more sensitive than EM; however, EM examination can produce direct evidence of the viral agent's presence in the target tissue and provide information for an understanding of the mechanism of viral pathogenesis.
FIG. 3. Virus isolated from urine specimens. A. Thin section of cytomegalovirus in infected cells, illustrating a characteristic intranuclear inclusion (NI), and mature virus particles in cytoplasmic vacuoles. Bar = 1.0 μm. B. Papovavirus in the nucleus of an infected cell. Bar = 0.5 μm. Insert shows the characteristic structure of papovavirus in a negative-stained preparation. Bar = 0.1 μm.
FIG. 4. A. Herpesvirus nucleocapsids in the nucleus of an infected brain cell from a biopsy specimen. Bar = 0.5 μm.  B. Adenovirus particles in crystalline arrays in the nucleus of an infected liver cell from an autopsy specimen. Bar = 0.5 μm.
**Observation of Virus from Lung, Liver, and Other Organs**

Adenovirus is often isolated from lung and other organs of immunocompromised patients. Adenovirus-infected cells in various tissues show intranuclear inclusions when examined under the light microscope. Adenovirus inclusions can be mistaken for CMV inclusions. To distinguish CMV from adenovirus, EM examination is the preferred method. One of our early experiences in the diagnosis of adenovirus infection occurred when a chronic myelogenous leukemia patient, who had received a bone marrow transplant, developed a rapidly fatal gastroenteritis. Histopathologic examination by light microscopy revealed what looked like CMV intranuclear inclusions in the cells of liver, lungs, and small bowel tissues and was diagnosed as CMV infection by pathologists. Postmortem specimens were also processed for EM and virus isolation in cell cultures. EM examination revealed virus particles that resembled adenovirus but not CMV in the nuclei of infected liver cells (Fig. 4B). Virus isolation showed that this isolate had a broad spectrum of cell susceptibility; it replicated in many types of cell cultures, including primary guinea pig embryo cells, resembling that property of herpes simplex virus (HSV) \([43,44]\). By tissue-culture neutralization test, however, the final identification of this virus isolate was adenovirus type 2. In this case, EM identification was the key to an accurate diagnosis of this adenovirus infection.

**Virus Observed in Skin Tumors**

Human papilloma virus (HPV), the etiology of common warts, was first detected through use of the electron microscope by Strauss et al. in 1949 \([45]\) and later confirmed by others \([46–48]\). HPV belongs to the papovavirus group, with a diameter of 50–55 nm, and exhibits icosahedral symmetry. It propagates in certain epidermal cells in human common warts and appears as intranuclear crystalline arrays \([45,47]\) (Fig. 5A). At present, HPV has never been propagated in any cell culture *in vitro*. For a long time, EM examination has been the only method of detecting the presence of HPV in laboratory diagnosis. Recently, nucleic acid hybridization, using an HPV DNA probe, has become commercially available for laboratory diagnosis of HPV infection.

Molluscum contagiosum virus (MCV) is a poxvirus causing skin tumor. The virus can only be detected by EM examination (Fig. 5B). Similar to HPV, MCV is not capable of replicating in cell culture *in vitro*; EM is the method used for laboratory diagnosis \([48]\).

**Identification of Isolates with Unusual Properties**

In a routine diagnostic virology laboratory, most of the virus isolates can be identified by their biological properties, such as the appearance of cytopathic changes, hemagglutination activity to avian or mammalian red blood cells, or by antigenic properties, using group- and type-specific antiserum. In some cases, however, identification of isolates possessing unusual properties can be difficult. EM has often provided rapid identification of those viruses with unusual properties.

**Identification of Unusual HSV Isolates by EM**

HSV is the most common virus isolate encountered in clinical specimens today. Since the availability of monoclonal antibody for typing of HSV-1 and HSV-2 by immunofluorescence assay (IFA), the identification of HSV isolates has become a simpler and easier task than ever before. Once the characteristic CPE of HSV is
observed, confirmatory typing by IFA can be made immediately. In our experience during the last two and a half years, however, among 682 HSV isolates, there were two HSV isolates which were untypable by use of monoclonal antibody to HSV-1 and HSV-2. These two isolates were finally identified by EM and confirmed by IFA, using polyclonal antibody to HSV. According to EM observation, one of the HSV isolates was type 2, based on the presence of intranuclear fiber structures similar to the characteristics of HSV-2 structure (Fig. 6) previously reported [49,50]. This result
FIG. 6. Herpes simplex virus type 2 identified by EM. Note viral nucleocapsids (NC) and characteristic fiber structures (F) in the nucleus of an infected cell. Bar = 0.2 μm.

was reconfirmed by use of the selective inhibition of (E)-5(2-bromovinyl)-2'-deoxyuridine [51]. In cases of HSV isolates that are not identifiable by monoclonal antibody to either type of HSV by IFA, EM would be very useful as a supplemental method for HSV identification.

Detection of Endogenous Agents in Cell Culture

For virus isolation, clinical specimens are routinely inoculated into susceptible cell-culture systems. Primary cell cultures derived from monkeys or other animals often harbor viruses of their own. Primary monkey kidney cells frequently contain simian virus type 5 (SV-5), simian virus type 40 (SV-40), herpesvirus, cytomegalovirus, and adenovirus of simian origin [52]. It is important that a virologist be aware of the presence of such endogenous viruses in cell cultures being used for clinical specimens.

EM has been used frequently for rapid identification of endogenous agents in cell cultures and calf serum [53,54]. An example is illustrated in Fig. 7: a mixed infection of SV-40 and SV-5 in a monkey kidney cell, which was originally inoculated with SV-5 only, a parainfluenzavirus. SV-40 was apparently an endogenous virus in the cell cultures used for growing SV-5.

McCoy cells, a cell line of mouse origin, are widely used in many laboratories for cultivation of Chlamydia trachomatis. This cell line was found to contain C-type virus particles by EM observation (Fig. 8) [Fong CKY: unpublished observation].

Mycoplasma is another common contaminant in cell culture and in calf serum.
FIG. 7. A primary monkey kidney cell infected with both SV-5 and SV-40. Worm-like nucleocapsids of SV-5 are present in the cytoplasm (arrows), and virions of SV-40 can be observed in the nucleus. SV-40 is an endogenous virus from monkey kidney cells. Bar = 0.2 μm.

FIG. 8. An endogenous murine C-type virus in a McCoy cell culture; several virus particles are in the process of budding from the cell membrane. Bar = 0.5 μm. Insert is a higher magnification of a budding virus particle. Bar = 0.1 μm.
Although mycoplasma can be detected by cultivation in special medium [55] or by DNA staining followed by fluorescent microscopic examination [56], EM examination of thin sections of contaminated cell cultures often provides a rapid and reliable identification (Fig. 9).

CONCLUDING REMARKS

It is beyond doubt that the application of EM in diagnostic virology has had a great effect on the rapid and accurate laboratory diagnosis of viral infections in certain clinical diseases. In specimens containing a large number of virus particles, such as vesicular fluid and stool extracts, EM examination of negative-stained preparations can provide a rapid diagnosis within an hour. In specimens with a low virus yield such as urine, various methods of concentration can be applied to the specimens and the diagnosis obtained within two hours. For virus isolates with unusual biologic and antigenic properties or unknown etiology, EM can provide a rapid identification of the virus group based on morphological characteristics. For those viruses which cannot be cultivated in cell cultures, EM is the method of choice for their detection. Endogenous viral agents and/or mycoplasma contaminants in cell cultures often produce adverse effects on the routine use of cell cultures for clinical specimens. By employing EM, rapid detection of such contaminants is possible.

In spite of its limitation of low sensitivity, EM has a definite role in the rapid, accurate diagnosis of many interesting clinical diseases of viral etiology. In some instances, EM is the only method of choice.

DEDICATION

This paper is dedicated to Professor G.D. Hsiung on her seventieth birthday. To me, Professor Hsiung is a mentor, an understanding co-worker, and a long-time friend whose continuous inspiration has led me into the field of diagnostic virology, electron microscopy, and research in the diagnosis of human viral diseases.
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REFERENCES

1. Chernesky MA: The role of electron microscopy in diagnostic virology. In Diagnosis of Viral Infections. Edited by D Lennette, S Specter, KD Thompson. Baltimore, University Park Press, 1979, pp 125–142
2. Doane FW, Anderson N: Electron and immune electron microscopic procedures for diagnosis of viral infections. In Comparative Diagnosis of Viral Diseases II. Edited by E Kurstak, C Kurstak. New York, Academic Press, 1977, pp 505–539
3. Doane FW, Anderson N, Zbitnew A, Rhodes AJ: Application of electron microscopy to the diagnosis of virus infections. Can Med Assoc J 100:1043–1049, 1969
4. Doane FW, Anderson N: Electron Microscopy in Diagnosis Virology. A Practical Guide and Atlas. New York, Cambridge University Press, 1987
5. Hsiung GD, Fong CKY, August MJ: The use of electron microscopy for diagnosis of virus infections: An overview. Progr Med Virol 25:133–159, 1979
6. Brenner S, Horne RW: A negative staining method for high resolution electron microscopy of viruses. Biochim Biophys Acta 34:103–110, 1959
7. Almeida JD: Practical aspects of diagnostic electron microscopy. Yale J Biol Med 53:5–18, 1980
8. Hammond GW, Hazelon PR, Chuang I, Klisko B: Improved detection of viruses by electron microscopy after direct ultracentrifugation preparation of specimens. J Clin Microbiol 14:210–221, 1981
9. Kellenberger E, Arber W: Electron microscopical studies of phage multiplication. I. A method of quantitative analysis of particle suspensions. Virology 3:245–255, 1957
10. Anderson N, Doane FW: Agar diffusion method for negative staining of microbial suspensions in salt solutions. Appl Microbiol 24:495–496, 1972
11. Doane FW: Identification of viruses by immunoelectron microscopy. In Viral Immunodiagnosis. Edited by E Kurstak, R Morisset. New York, Academic Press, 1974, pp 237–256
12. Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM: Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis, J Virol 10:1075–1081, 1972
13. Feinestone SM, Kapikian AZ, Purcell RH: Hepatitis A: Detection by immune electron microscopy of a virus like antigen associated with acute illness. Science 182:1026–1028, 1973
14. Katz D, Straussman Y, Shahar A, Kohn A: Solid-phase immune electron microscopy (SPIEM) for rapid viral diagnosis. J Immunol Meth 38:171–174, 1980
15. Howe C, Bach T, Hsu KC: Application of immunoferritin techniques for the detection of viral and cellular antigens. In Viral Immunodiagnosis. Edited by E Kurstak, R Morisset. New York, Academic Press, 1974, pp 215–234
16. Hopley JFA, Doane FW: Development of a sensitive protein A-gold immunoelectron microscopy method for detecting viral antigens in fluid specimens. J Virol Meth 12:135–147, 1985
17. Doane FW, Anderson N, Chao J, Noonan A: Two-hour embedding procedure for intracellular detection of viruses by electron microscopy. Appl Microbiol 27:407–410, 1974
18. Sabatini DD, Bensch K, Barrnett RJ: Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J Cell Biol 17:19–58, 1963
19. Hayat MA: Principles and Techniques of Electron Microscopy: Biological Application, Volume 1. Baltimore, MD, University Park Press, 1981
20. Nagler FPO, Rake G: The use of the electron microscope in diagnosis of variola, vaccinia, and varicella. J Bact 55:45–51, 1948
21. Van Rooyen CE, Scott GD: Smallpox diagnosis with reference to electron microscopy. Can J Public Health 39:467–477, 1948
22. Flewett TH, Bryden AS, Davies H: Virus particles in gastroenteritis. Lancet ii:1497, 1973
23. Bishop RF, Davidson GP, Holmes IH, Ruck BJ: Detection of a new virus by electron microscopy of faecal extracts from children with acute gastroenteritis. Lancet i:149–151, 1974
24. Bradburne AF, Almeida JD, Gardner PS, Moosai RB, Nash AA, Coombs RR: A solid-phase system (SPACE) for the detection and quantification of rotavirus in faeces. J Gen Virol 44:615–623, 1979
25. Brandt CD, Kim HW, Rodriguez WJ, Thomas L, Yolken RH, Arrobiio JO, Kapikian AZ, Parrott RH, Chanock RM: Comparison of direct electron microscopy, immune electron microscopy, and rotavirus

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enzyme-linked immunosorbent assay for detection of gastroenteritis viruses in children. J Clin Microbiol 13:976–981, 1981
26. Hung T, Chen G, Wang C, Yao H, et al: Waterborne outbreak of rotavirus diarrhea in adults in China caused by a novel rotavirus. Lancet i:1139–1142, 1984
27. Gary GW Jr, Hierholzer JC, Black RE: Characteristics of noncultivable adenoviruses associated with diarrhea in infants: A new subgroup of human adenoviruses. J Clin Microbiol 10:96–103, 1979
28. Yolken RH, Lawrence F, Leister F, Takiff HE, Strauss SE: Gastroenteritis associated with enteric type adenovirus in hospitalized infants. J Pediat 101:21–26, 1982
29. Uhnno I, Wadell G, Svennson L, Johansson ME: Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and children. J Clin Microbiol 20:365–372, 1984
30. Kapikian AZ, Dienstag JL, Purcell RJ: Immune electron microscopy as a method for the detection, identification, and characterization of agents not cultivable in an in vitro system. In Manual of Clinical Immunology. Edited by NR Rose, H Friedman. Washington, D.C., American Society for Microbiology, 1976, pp 467–480
31. Barron RC, Murphy FD, Greenberg HB, et al: Norwalk gastrointestinal illness: An outbreak associated with swimming in a recreational lake and secondary person-to-person transmission. Am J Epidemiol 115:163–172, 1982
32. Grohmann GS, Greenberg HB, Welch BM, Murphy AM: Oyster-associated gastroenteritis in Australia: The detection of Norwalk virus and its antibody by immune electron microscopy and radioimmunossay. J Med Virol 6:11–19, 1980
33. Gunn RA, Janowski HT, Lieb S, Prather EC, Greenberg HB: Norwalk virus gastroenteritis following raw oyster consumption. Am J Epidemiol 115:348–351, 1982
34. Stagno S, Pass RF, Dworsky ME, Alford CA: Congenital and perinatal cytomegalovirus infections. Semin Perinatol 7:31–42, 1983
35. Henry C, Hartsock RJ, Kirk Z, Behrer R: Detection of viruria in cytomegalovirus-infected infants by electron microscopy. Am J Pathol 69:435–439, 1978
36. Lee FK, Nahmias AJ, Stagno S: Rapid diagnosis of cytomegalovirus infection in infants by electron microscopy. N Engl J Med 299:1266–1270, 1978
37. Montplaisir S, Belloncik S, Leduc NP, Onji PA, Martineau B, Kurstak E: Electron microscopy in the rapid diagnosis of cytomegalovirus: Ultrastructural observation and comparison of methods of diagnosis. J Infect Dis 125:533–538, 1972
38. Geaves CA, Smith TF, Shuster EA, Pearson GR: Rapid detection of cytomegalovirus in MRC-5 cells inoculated with urine specimens by using low-speed centrifugation and monoclonal antibody to an early antigen. J Clin Microbiol 19:917–919, 1984
39. Gardner SD, Field AM, Coleman DV, Hulme B: New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet i:1253–1257, 1971
40. Dougherty RM, DiStephano HS: Isolation and characterization of a papovavirus from human urine. Proc Soc Exp Biol Med 146:481–487, 1974
41. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH: Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. Lancet i:1257–1260, 1971
42. Sharer LR, Epstein LG, Cho E-S, et al: Pathologic features of AIDS encephalopathy in children: Evidence for LAV/HTLV-III infection of brain. Hum Pathol 17:271–284, 1986
43. Landry ML, Fong CKY, Neddermann K, Solomon L, Hsiung GD: Disseminated adenovirus infection in an immunocompromised host: Pitfalls in diagnosis. Am J Med 83:555–559, 1987
44. Fong CKY, Landry ML, Hsiung GD: Replication of human adenovirus in guinea pig embryo cells: An ultrastructural study. Arch Virol 93:247–260, 1987
45. Strauss MJ, Shaw EW, Bunting H, Melnick JL: “Crystalline” virus-like particles from skin papillomas characterized by intranuclear inclusion bodies. Proc Soc Exp Biol Med 72:46–50, 1949
46. Bunting H: Close-packed array of virus-like particles within cells of a human skin papilloma. Proc Soc Exp Biol Med 84:327–332, 1953
47. Williams MG, Howatson AF, Almeida JD: Morphological characterization of the virus of the human common wart (verruca vulgaris). Nature 189:895–897, 1961
48. Melnick JL, Bunting H, Banfield WG, Strauss MJ, Gaylord WH: Electron microscopy of viruses of human papilloma, molluscum contagiosum, and vaccinia, including observations on the formation of virus within the cells. Ann NY Acad Sci 54:1214–1225, 1952
49. Couch EF, Nahmias AJ: Filamentous structures of type 2 Herpesvirus hominis infection of the chorioallantoic membrane. J Virol 3:228–232, 1969
50. Young SK, Rowe NH, Sanderlin KC: Herpes simplex virus types 1 and 2 in clinical infections: Differences observed by electron microscopy. J Infect Dis 135:486–489, 1977
51. Mayo DR: Differentiation of herpes simplex virus types 1 and 2 by sensitivity to (E)-5(2-bromovinyl)-2’-deoxyuridine. J Clin Microbiol 15:733–736, 1982
52. Hsiung GD: Latent virus infections in primate tissues with special reference to simian viruses. Bacteriol Rev 32:185–205, 1968
53. Anderson N, Doane FW: Microscopic detection of adventitious viruses in cell cultures. Can J Microbiol 18:299–304, 1972
54. Fong CKY, Gross PA, Hsiung GD, Swack NS: Use of electron microscopy for detection of viral and other microbial contaminants in bovine sera. J Clin Microbiol 1:219–224, 1975
55. Barile MF, et al: The identification and sources of mycoplasmas isolated from contaminated cell cultures. Ann NY Acad Sci 225:251–264, 1973
56. Chen TR: In situ detection of mycoplasma contamination in cell cultures by fluorescent Hoechst 33258 stain. Exp Cell Res 104:255–262, 1977