Virus-Like Particles and Cytopathic Activity in Urine of Patients with Leukemia


In 1951 Gross provided evidence that leukemia in mice was of viral etiology.1 Subsequently more than a dozen different forms of rodent leukemia have been found to be transmissible by cell-free filtrates.2 In each form of murine leukemia a consistent type of subcellular particle has been seen by electron microscopy in the acellular extracts, and these are considered to mediate the disease.3 These experimental studies have prompted and conditioned the search for viruses in human leukemia. Indeed, several workers have detected virus-like particles similar to those seen in the rodent leukemias in the plasma,4-10 lymph nodes,11-13 and bone marrow14 of some patients with acute and chronic forms of leukemia, lymphomas, Hodgkin’s disease, and infectious mononucleosis. When the thin sectioning technic was employed, the percentage of patients exhibiting such particles in plasma pellets has varied from 14 to 25 per cent.4-6 By negative staining methods, with or without density gradient concentration, the proportion of patients with plasma particles has ranged from 50 to 100 per cent.6-10

Attempts to demonstrate biological activity in animals with material taken from patients with leukemia, although occasionally successful,15-19 have been of questionable significance because of the presence of latent oncogenic viruses in mice. Similarly, cytopathic changes in tissue culture inoculated with material derived from patients with leukemia,15,16,20-23 and tissue explants from these patients,11,24,25 have sometimes, but not always,26-29 suggested the presence of virus. Other workers have isolated mycoplasma organisms from the blood and the bone marrow of patients with acute leukemia.30-35 Thus, the nature of the agent from human sources mediating disease in animals and changes in tissue culture is unclear.

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Table 1.—Characteristics of Patients with Virus-Like Particles in Urine

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dx</th>
<th>Age and Sex</th>
<th>Time from Diagnosis (Days)</th>
<th>WBC $\times 10^3$</th>
<th>State of Disease</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. C.</td>
<td>ALL</td>
<td>2 M</td>
<td>2</td>
<td>15</td>
<td>Active</td>
<td>POMP</td>
</tr>
<tr>
<td>T. R.</td>
<td>ALL</td>
<td>3 F</td>
<td>3</td>
<td>3</td>
<td>Active</td>
<td>POMP</td>
</tr>
<tr>
<td>P. A.</td>
<td>ALL</td>
<td>11 M</td>
<td>595</td>
<td>6</td>
<td>Active</td>
<td>Off 2 weeks</td>
</tr>
<tr>
<td>R. K.</td>
<td>ALL</td>
<td>20 M</td>
<td>5</td>
<td>60</td>
<td>Active Off 2 weeks</td>
<td></td>
</tr>
<tr>
<td>C. H.</td>
<td>AGL</td>
<td>32 M</td>
<td>75</td>
<td>14</td>
<td>Active Off 2 weeks</td>
<td></td>
</tr>
<tr>
<td>S. L.</td>
<td>AGL</td>
<td>33 M</td>
<td>21</td>
<td>110</td>
<td>Active Off 2 weeks</td>
<td></td>
</tr>
<tr>
<td>J. B.</td>
<td>CGL</td>
<td>39 M</td>
<td>7</td>
<td>55</td>
<td>Active Remission</td>
<td></td>
</tr>
<tr>
<td>W. B.</td>
<td>CGL</td>
<td>21 M</td>
<td>364</td>
<td>77</td>
<td>Active Remission</td>
<td></td>
</tr>
<tr>
<td>S. F.</td>
<td>Uveitis</td>
<td>25 F</td>
<td>1021</td>
<td>15</td>
<td>Active Remission</td>
<td></td>
</tr>
</tbody>
</table>

ALL—Acute lymphocytic leukemia.
AGL—Acute granulocytic leukemia.
CGL—Chronic granulocytic leukemia.

In vitro assay and transformation systems will have to be developed before any relationship between these findings and human leukemia can be made. Since the development of such systems depends, in large measure, on the availability of test material, an investigation of urine was undertaken in an effort to find more convenient and readily accessible sources of material.

Materials and Methods

Sample Preparation

Urine samples from 16 patients with acute leukemia, 2 patients with chronic granulocytic leukemia and 1 patient with Burkitt’s lymphoma were studied. Clinical features of these patients are listed in Tables 1 and 2. Urine from 15 patients without leukemia served as control material. Three of the control patients had uveitis. Two of these were on methotrexate therapy and one was on low dosage corticosteroid treatment. Four of the controls had chronic nephritis with variable amounts of protein, erythrocytes, and leukocytes in the urine. One control subject was in the convalescent phase of infectious mononucleosis (heterophile antibody titer negative). The remaining 7 controls were normal, healthy subjects. Two were parents of a patient with acute leukemia (R. S., Table 2). One of the normal, healthy controls worked with murine leukemia viruses, and two worked with specimens from patients with leukemia.

Thirty urine specimens from patients with leukemia and 17 urine samples from the control group were examined. The volume of urine used to form pellets in the group with leukemia ranged from 25 cc. to more than 3 liters (average volume 460 cc.). Urine volume in controls ranged from 20 to 500 cc. (average volume, 215 cc.). Large urine specimens were reduced to 20-100 cc. volumes by ultrafiltration at 4 C. Most urine and plasma specimens were stored at -20 C. until processed.

Differential centrifugation for partial purification was conducted at forces of 1000 g and 2500 g for 10-20 minutes. Pelletization was carried out in a Spinco Model L ultracentrifuge at 44–105,000 g for one hour using a 21, 30, or 40 fixed angle rotor or a SW 39L swinging bucket rotor.

When plasma was studied, venous blood samples ranging in volume from 5 to 30 cc.
### Table 2.—Characteristics of Patients Negative for Virus-Like Particles in Urine

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dx</th>
<th>Age and Sex</th>
<th>Time from Diagnosis (Days)</th>
<th>WBC x 10⁶</th>
<th>Highest State of Disease</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. E.</td>
<td>ALL</td>
<td>3 F</td>
<td>167</td>
<td>15</td>
<td>15 Active</td>
<td>Prednisone</td>
</tr>
<tr>
<td>M. W.</td>
<td>ALL</td>
<td>5 M</td>
<td>296</td>
<td>750</td>
<td>7 Active</td>
<td>Off 4 days</td>
</tr>
<tr>
<td>J. E.</td>
<td>ALL</td>
<td>5 M</td>
<td>103</td>
<td>6</td>
<td>5 Active Methotrexate</td>
<td>Prednisone</td>
</tr>
<tr>
<td>R. H.</td>
<td>ALL</td>
<td>5 M</td>
<td>259</td>
<td>618</td>
<td>55 Active Cytosine</td>
<td>Arabinoside</td>
</tr>
<tr>
<td>K. B.</td>
<td>ALL</td>
<td>9 M</td>
<td>23</td>
<td>14</td>
<td>1.5 Active</td>
<td>Off 5 days</td>
</tr>
<tr>
<td>J. S.</td>
<td>ALL</td>
<td>10 M</td>
<td>389</td>
<td>810</td>
<td>1 Active Off 3 days</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>R. S.</td>
<td>ALL</td>
<td>18 M</td>
<td>127</td>
<td>120</td>
<td>77 Active</td>
<td>Prednisone</td>
</tr>
<tr>
<td>C. V. R.</td>
<td>ALL</td>
<td>18 M</td>
<td>502</td>
<td>80</td>
<td>1 Active Radiation</td>
<td></td>
</tr>
<tr>
<td>J. K.</td>
<td>AAI</td>
<td>37 F</td>
<td>-20</td>
<td>2.7</td>
<td>1.5 Active</td>
<td>None</td>
</tr>
<tr>
<td>M. B.</td>
<td>CGL</td>
<td>26 F</td>
<td>1221</td>
<td>670</td>
<td>0.7 Active</td>
<td>Prednisone</td>
</tr>
<tr>
<td>D. A.</td>
<td>BLI</td>
<td>10 M</td>
<td>30</td>
<td>8</td>
<td>8 Active</td>
<td>None</td>
</tr>
</tbody>
</table>

*AML—acute monocytic leukemia.
†Aplastic anemia—acute lymphocytic leukemia first diagnosed 20 days after this study.
‡BL—Burkitt’s lymphoma.

were collected in a half-volume of 0.6 M potassium citrate anticoagulant. The plasma was usually separated from cells immediately and processed like the urine specimens. Seventeen plasma samples from 6 patients with leukemia, 1 sample from a normal, healthy subject, and 1 sample from a patient with multiple myeloma were studied.

### Density Gradient

Density gradient studies using 0.5 cc. of resuspended urine pellet material were carried out in linear potassium citrate gradients using either an SW25 (25 ml.) or SW39L (4.6 ml.) Spinco rotor at 54,000 to 105,000 g. The gradients were unloaded either by puncturing the bottom of the tube and collecting fractions dropwise or by puncturing the side of the tube and collecting fractions dropwise or by puncturing the side of the tube and collecting 0.1 cc. fractions in a tuberculin syringe at 0.5 cm. intervals along the length of the tube. Refractive indices were determined at 30 C. and converted to density by reference to a standard density curve for saturated potassium citrate buffer (pH 6.75) in Dulbecco’s phosphate-buffered saline solution. Fractions containing bands were dialyzed overnight against a series of progressively more dilute potassium citrate buffer solutions ending in a final concentration of 0.05 M and then repelleted.

### Electron Microscopy

Urine pellets and tissue culture cells were fixed in Dalton’s chrome osmium solution for 45-90 minutes at 4 C., washed once an stored in 10 per cent formalin overnight prior to graded dehydration and embedment in methacrylate or an epomaraide mixture. Occasionally, tissue culture cells were fixed in 5 per cent glutaraldehyde in phosphate or cacodylate buffer, pH 7.4, for 15-120 minutes before placing in Dalton’s chrome osmium solution. When treated in this fashion, 10 per cent formalin was not used. Thin sections were cut with diamond knives on an LKB ultramicrotome, placed on collodion-carbon coated copper grids, and stained with uranyl acetate and lead citrate for 5-60 minutes. Sections were examined in an RCA EMU 3C electron microscope at an accelerating voltage of 50. Objective aperture size was 25-50 microns.

Thin sections of pellets were scanned for a minimum of 20 minutes. If particulate matter was present, a more prolonged search was undertaken. Particulate matter occurred more frequently in patients with leukemia.
Tissue Culture Studies

Five urine and 10 plasma specimens from patients with leukemia were cultured on 3 lines of tissue culture cells: rhesus monkey kidney, Hep-2 human epithelial cells, and WI-38 fibroblasts. One plasma and 3 urine samples from control patients were also cultured on these 3 cell lines. Eight urine specimens from patients with leukemia and 4 urine samples from controls were cultured on WI-38 fibroblasts alone. Inoculation of tissue culture cells was carried out by placing 0.2 cc. of urine or plasma on the cells overnight after removing the tissue culture medium which consisted of Eagle's #2 medium containing penicillin and streptomycin and 5 per cent horse serum. Tissue cultures were observed for 2 to 5 weeks. Tissue culture medium was changed biweekly.

Four urine samples and 9 plasma samples from patients with leukemia, and 1 plasma and 3 urine specimens from control patients, were inoculated directly onto PPLO agar and broth. The PPLO medium was enriched with yeast extract and horse serum and contained penicillin and thallium acetate. These cultures for mycoplasma were incubated both aerobically and anaerobically at 37°C. All tissue culture cells showing cytopathic effects and suitable control cells were cultured for mycoplasma organisms by a slightly modified method utilizing several enrichment substances and without antibiotics.*

RESULTS

Electron Microscopy

Virus-like particles were observed in the urine specimens of 7 of 16 patients with acute leukemia, in 1 of 2 patients with chronic granulocytic leukemia, and in 1 of 15 control patients. Similar particles were observed in the plasma of 1 patient with acute lymphocytic leukemia and in 1 patient with acute monocytic leukemia. Examples of these double-membraned particles are shown in Figure 1A-E. The particle size ranged from 65-125 μm in diameter. The diameter of the internal ring averaged 40-70 μm.

The clinical features of the patients who had virus-like particles in their urine are listed in Table 1. The presence of particles did not correlate with age, type, stage, or duration of leukemia, or height of the white blood cell count. Particles were found most frequently when patients were on an intensive form of combination chemotherapy, termed POMP. This therapy consisted of the simultaneous parenteral administration of 4 active antileukemic agents: vincristine, 6-mercaptopurine, methotrexate, and prednisone. Particles were also seen before therapy and after its discontinuation. One of the 3 patients studied in remission (J. B.) was repeatedly positive. The virus-like particles illustrated in Figure 1A-E are of two morphologic types. One type of particle contains either no nucleoidal material (Fig. 1A,E) or a dense accumulation of nucleoidal substance (Fig. 1C). The membranes of these particles are concentric. In the other type of particle (Fig. 1B,D) the nucleoidal substance is dispersed within the inner viral membrane. The external membrane often forms an irregular envelope around the circular inner membrane. Both types of particles were observed in patients with the various forms of leukemia.

The clinical features of the patients with leukemia in whom no particles were seen were similar to those who had particles in their urine (Table 2).

*Cultures for mycoplasma organisms were obtained through the cooperation of Dr. M. F. Barile, Division of Biological Standards, and Dr. Ewdokia Ryschenkow, Microbiology Service, Clinical Center, National Institutes of Health.
Fig. 1.—A. Virus-like particle seen in urine of a patient (R. K.) with acute lymphocytic leukemia during therapy. Particles shown in A-E show the three morphologic criteria used in this study: (a) There is a double-membraned ring; (b) the inner membrane is more electron-dense than the outer ring; (c) the particle stains more intensely than the surrounding material. Magnification of A-E is reduced 20 per cent from approximately 200,000X.

B. Particle from urine of patient (S. L.) with acute granulocytic leukemia. This particle had banded at density 1.17 Gm. cc. in a potassium citrate gradient. Nonspecific background material is present, indicating that this gradient technic does not remove this material.

C. Particle in urine pellet of a patient (W. B.) with chronic granulocytic leukemia.

D. This virus-like particle appeared in the urine of a patient (J. B.) with acute granulocytic leukemia in remission. The extension of the outer coat of this particle is suggestive of a tail.

E. Virus-like particle in urine of patient R. K. has two closely apposed inner membranes and a dense outer membrane. This particle resembles most closely the morphology of cytomegalovirus in its intranuclear phase.
Table 3.—Vir  

<table>
<thead>
<tr>
<th>Type of Acute Leukemia</th>
<th>Number of Patients</th>
<th>Number of Simultaneous Studies</th>
<th>Urine Positive</th>
<th>Plasma Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytic</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Granulocytic</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Monocytic</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>4</strong></td>
<td><strong>10</strong></td>
<td><strong>6</strong></td>
<td><strong>4</strong></td>
</tr>
</tbody>
</table>

These patients, however, were either not receiving treatment or were on more conventional doses of antileukemic therapy.

Plasma and urine samples collected within 24 hours of each other were studied on 10 occasions in 4 patients with leukemia (Table 3). Particles were found in the urine on 6 occasions and in the plasma on 4.

One (S. F.) of 15 control patients was positive for virus-like particles (Table 1). This patient was on intermittent parenteral methotrexate therapy for uveitis. When restudied while still on methotrexate therapy, this patient was negative. A second patient with uveitis on low-dosage corticosteroid therapy was negative on two occasions.

**Density Gradient Studies**

Five resuspended urine pellets in 3 patients with acute leukemia (S. L., J. B., R. K.) and one urine pellet from a patient with chronic granulocytic leukemia (W. B.) were centrifuged through linear potassium citrate gradients. A band was always observed at 2.4–2.6 cm. from the top of the 5-cc. gradient tube (Fig. 2). Virus-like particles could be found in this band, density 1.17 Gm./cc. after repelletization and examination in thin section. On 4 occasions a band was also present at 2.0–2.2 cm. from the top of the tube, and in 2 of these instances (patients S. L. and W. B.) virus-like particles were seen at this level, density approximately 1.13 Gm./cc.

**Tissue Culture Studies**

All urine and plasma specimens from patients with leukemia and controls showed no effect on the 3 tissue culture lines after 3 blind passages, each passage being performed at weekly intervals and observed for 10–14 days. When cultured on fibroblasts alone, 2 urine specimens from patients with leukemia were negative when observed for only 2 weeks. However, 4 or 6 urine specimens from 2 adult patients with acute leukemia (J. B. and R. K.), and 1 urine specimen of a patient with chronic granulocytic leukemia (W. B.), showed cytopathic effects on WI-38 fibroblasts when observed for 30 days. The cytopathic effects (Fig. 3) generally appeared between the 4th to 25th days after inoculation, developed focally, and spread to involve the entire culture in a manner suggestive of a cytomegalovirus infection. The cytopathic effect persisted after 4 passages in 1 instance and 2 passages in all others. Cytopathic effects appeared most regularly when freshly voided urine was cultured. In one instance, however, a urine sample which had been frozen to −20 C. and
thawed repeatedly over a 5-month period produced cytopathic effects in the first blind passage. A virus-like particle seen in the urine of this patient (R. K.) is shown in Figure 1E. This closely resembles cytomegalovirus morphologically.

**Cultures for Mycoplasma**

All of 14 urine and 3 plasma specimens from patients with leukemia and from controls were negative on direct culture for mycoplasma. When media from 4 tubes of WI-38 fibroblasts showing cytopathic effects were cultured for mycoplasma, all were positive. In addition, 1 of 3 control uninoculated tubes of WI-38 fibroblasts was also positive for a mycoplasma organism. This control tube did not show cytopathic effects. When the mycoplasma species from a tube showing cytopathic effects was reinoculated onto WI-38 fibroblasts, no cytopathic effect developed after 5 weeks of observation.

**Electron Microscopy of Tissue Culture Cells**

Three tissue cultures showing widespread cytopathic effects were examined
Fig. 3.—Cytopathic effects in WI-38 fibroblasts produced by urine from a patient (R. K.) with acute lymphocytic leukemia. Control fibroblasts are shown above (150).

by electron microscopy, and virus-like particles were observed in one of these (patient W. B.). In this culture particles were present in only a few cells. Figure 4 shows the nucleus of an infected cell. Two forms of virus-like particle can be distinguished. One type has a double-membrane ring. The other type, exemplified by a particle at the lower left of the figure (indicated by the short arrow), has a single limiting membrane with an eccentric nucleoid. Figure 5 shows particles which appear to be outside the cell. These particles were not seen in control fibroblasts. These particles are morphologically similar to those seen in the urine of patients with leukemia as shown in Figure 1A. C. E.

DISCUSSION

This study presents morphologic evidence that virus-like particles are present in the urine of patients with acute and chronic forms of leukemia. As shown in Table 3, the particles are present as frequently in urine as in plasma. Most patients who exhibited particles were receiving intensive antileukemia chemotherapy. While a priori one might think that particles would be less frequent during a treatment, this finding suggests that particles may be even more frequent in this circumstance, possibly due to rapid cell lysis. Increased numbers of particles during therapy have also been observed in the plasma of patients with leukemia by other workers. The morphology of these particles resembles that of the type C particle seen extracellularly and in cytoplasmic...
VIRUS-LIKE PARTICLES IN LEUKEMIA

Fig. 4.—A portion of the nucleus of a WI-38 fibroblast from a culture inoculated with urine of a patient with chronic granulocytic leukemia (W. B.). Nuclear membrane can be seen at extreme right. Some of the virus-like particles scattered throughout the nucleus are indicated by arrows.

vacuoles in the murine leukemias. Furthermore, their sedimentation density in a potassium citrate gradient approximates that of the Rauscher murine leukemia virus.36

The one control patient who exhibited virus-like particles was on methotrexate therapy. Two other studies conducted during methotrexate therapy in controls and 6 studies of patients with leukemia on various types of antileukemia therapy including antimetabolites and radiation were negative, indicating that cytotoxic agents per se do not produce artefacts resembling virus-like particles.

The cytopathic effects observed in tissue culture following the inoculation of urine from 3 leukemic patients were consistent with cytomegalovirus activity. Herpes virus can also infect this cell line. However, inoculation of tissue culture cells showing the cytopathogenic effect into the chorioallantoic membrane of embryonated eggs was negative in 2 instances, providing evidence that Herpes virus was not causing the effect.
Fig. 5.—These virus-like particles in extracellular space of a tissue culture of fibroblasts inoculated with urine from patient W. B. resemble morphologically the particles seen in Figure 1A, C, E. A portion of fibroblast is present at bottom of figure.

Although the morphology and density characteristics of the virus-like particles resembled the type C particles of murine leukemia, these particles could not be clearly distinguished on morphologic grounds from the intranuclear forms of cytomegalovirus (Fig. 4). Furthermore, similar particles which appear to lie in the extracellular space (Fig. 5) may represent intranuclear forms of cytomegalovirus released when the cell ruptures. The virus-like particles in the urine could represent cytomegalovirus released by a similar mechanism. However, it is also possible that 2 or more viruses are present in urine of patients with leukemia, as suggested by the morphologic differences observed among the particles in this study.

It is of interest that these intranuclear virus-like particles resemble those seen in nuclei of cultured Burkitt tumor and American lymphoma cells. The particles from the Burkitt cells, however, do not cause cytopathic effects in WI-38 fibroblasts.

Cytomegalovirus has been cultured from urine of patients with leukemia by
VIRUS-LIKE PARTICLES IN LEUKEMIA

475

It has also been found in the urine of healthy children, indicating a widespread distribution of this virus. Its presence in adults with hematologic disorders and other debilitating conditions suggests that reactivation of a latent infection or acquisition of a new infection is not infrequent in these circumstances; thus, viruria would not be unexpected.

Better analytic methods are necessary to determine the true nature of these virus-like particles. Clearly, electron microscopy alone is not a sufficient test of homogeneity. Until purity can be assured, it will not be possible to attribute a particular activity to a specific morphologic entity. Progress in this area will require a carefully integrated approach using all the analytic preparative techniques available.

Summary

Urine pellet material of patients with leukemia was examined by electron microscopy in an effort to find more convenient sources of virus material for tissue culture and animal infectivity studies. Thirty urine specimens from 16 patients with acute leukemia, 2 patients with chronic granulocytic leukemia and 1 patient with Burkitt’s lymphoma, and 17 urine samples from 15 control patients were examined. Virus-like particles were observed in 7 of the 16 patients with acute leukemia, 1 of the 2 patients with chronic granulocytic leukemia, and 1 of the 15 controls. Although these particles showed slight morphologic differences, many were similar in ultrastructure and in density gradient characteristics to the virus particles observed in murine leukemia and to the particles seen by other workers in plasma and tissues of patients with leukemia and lymphoma.

Cytopathic effects on WI-38 fibroblasts were observed in urine from 3 patients with leukemia. These effects resembled cytomegalovirus infection, but confirmatory tests were not carried out. Whether the virus-like particles demonstrated by electron microscopy in this study are responsible for the cytopathic effects is at present uncertain. The correlation of a morphologic structure with tissue culture activity cannot be answered until suitable in vitro assay and transformation systems are developed to purify and test these particles. This study suggests that urine may serve as a convenient source for the study and development of such test systems using the electron microscope to correlate structure with biologic activity.

Summario in Interlingua

Pellets urinari ab patientes con leucemia esseva examinate per microscopia electronic con le objectivo de trovar un plus convenibile fonte de material viral pro studios de histoculturacion e de infectivitate in animales. Trenta specimenes de urina ab 16 patientes con leucemia acute, 2 patientes con chronic leucemia granulocytic, e 1 patiente con lymphoma de Burkitt, si ben como 17 specimenes de urina ab 15 subjectos de controlo esseva examinate. Particulas virusoide esseva observate in 7 del 17 patientes con leucemia acute, in 1 del 2 patientes con chronic leucemia granulocytic, e in 1 del 15 subjectos de controlo. Ben que iste particulas monstrava leve differentias
morphologic, multes esseva simile in lor caracteristicas de ultrastructura e de densitate gradiente con le partucas viral observe in leucemia murin e al partucas describite per altere investigatores in le plasma e le tissues de pacientes con leucemia e lymphoma.

Effectos cytopathic super fibroblastos WI-38 esseva observate in le urina de 3 patientes con leucemia. Iste effectos resimilava infection cytomegaloviral, sed tests de confirmation non esseva effectuate. Si le partucas virusoide demonstrate per microscopia electronic in iste studio es responsabile pro le effectos cytopathic remane incerte a iste momento. Le correlation de un structura morphologic con le activitate histocultural non pote esser determine usque appropriate systemas de essayage e de transformation in vitro es disveloppate pro purificar e testar le partucas. Le presente studio suggestiona que urina pote servir como fonte convenibile pro le studio e le disveloppamento de tal systemas de testage utilisante le microscopio electronic pro correlacionar structura con activitate biologic.

ACKNOWLEDGMENT

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REFERENCES


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