NCI-H292 as an alternative cell line for the isolation and propagation of the human paramyxoviruses

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Summary. Primary rhesus monkey kidney (MK) cells have long been the cells of choice for isolation and propagation of the human paramyxoviruses (parainfluenza 1, 2, 3, 4A, 4B, and mumps). However, problems with the supply and cost of MK cells and the presence of endogenous viruses, including herpes B virus and SV-5, necessitated a search for an alternative cell line. Continuous cell cultures of human origin (L132, A-549, HuT-292, HEK, G-293, G-401, A-498, A-704, CAKI-1, RD) and simian origin (LLC-MK2, BSC-1, MA-104, Vero) were evaluated for their capacity to support the growth of the human paramyxoviruses, as followed by cytopathic effect, hemadsorption, hemagglutination, and EIA. NCI-H292 (HuT-292) human lung mucoepidermoid carcinoma cells (ATCC # CRL-1848) proved to be the most sensitive line for cultivating all serotypes and strains of the paramyxoviruses. These cells were also shown to be a suitable substitute for MK in primary isolation of paramyxoviruses from clinical specimens. RPMI-1640 with 1.5 μg/ml trypsin was the preferred maintenance medium; alternatively, Eagle’s MEM supplemented with 1.5 μg/ml trypsin and 0.1% ITS was satisfactory. NCI-H292 cells are a continuous line with excellent growth characteristics, although the genetic polyploidy of the cells may limit the number of passages of usable cells.

Introduction

The human paramyxoviruses are most readily isolated in primary rhesus monkey kidney (MK) cell cultures. In addition to sensitivity, MK cells are particularly valuable for paramyxovirus isolation because virus growth can be visualized both by cytopathology (CPE) and hemadsorption (HAd). In fact, all 5 human parainfluenzaviruses were first isolated in vervet or rhesus MK cells and mumps-virus was initially recovered in rhesus monkeys [5, 8, 10, 11, 18]. Parainfluenza

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types 1, 4A, and 4B are generally recoverable only in MK cells, while parainfluenza 2 and 3 and mumps viruses can be isolated in several human and simian cell lines [5, 9, 12, 16, 23].

MK cells, however, are currently fraught with severe problems for which adequate solutions are not evident. (a) The trapping of rhesus monkeys in India is discouraged to preserve the species in its native habitat. (b) Primates carry many endogenous viruses (adventitious agents), e.g., adenoviruses, herpesviruses (including CMV), papovaviruses, myxoviruses, and enteroviruses, which carry the risk of infecting laboratory workers handling the animals or their tissues. For example, the problem of workers contracting monkey B virus (Herpesvirus simiae), which is harmless to monkeys but usually fatal in man, is well known [7, 19]. (c) The kidneys of rhesus monkeys are sometimes latently infected with adenoviruses, enteroviruses, and herpesviruses, and almost always are infected with SV-5 (a myxovirus) and SV-40 (a papovavirus). Although these two agents are not known to infect man, they often compromise virus isolation and identification work, reagent production, and vaccine testing, and thus constitute a major built-in viral contamination problem for the laboratory. (d) Despite the establishment of breeding colonies in the U.S., the use of monkeys solely for their kidneys is now generally regarded as wasteful and cruel. (e) Monkeys are expensive to feed and house, and the preparation of primary cell cultures from them is a labor-intensive and costly endeavor.

Despite the utility of MK cells in the past, these problems necessitated a search for an alternative cell line for paramyxovirus isolation. In this study, we report the use of NCI-H292 (HuT-292) cells as a suitable substitute for MK cells in the primary isolation and laboratory cultivation of the human paramyxoviruses.

Materials and methods

Viruses

Prototype strains of the human paramyxoviruses were obtained from our reference virus collection. Although the earliest passages available were in MK cells, all but parainfluenza virus 4A and 4B had been adapted to embryonated eggs. Low passage material from the egg-adapted viruses was used as starting virus for this study because it was free of adventitious agents. A total of 82 isolates representing a wide time and place distribution also were obtained from our reference virus repository: 18 strains of parainfluenza 1, 19 of type 2, 15 of type 3, 8 of type 4A, 4 of type 4B, and 18 strains of mumps virus. They had been isolated in MK cells and stored at −100 °C since that time.

Cell cultures

The cell types chosen for this study (Table 1) were obtained in-house or from the American Type Culture Collection (ATCC, Rockville, MD). All cells were prepared weekly as tube cultures under EMEM/10% FBS or EMEM/7% FBS/1% ITS growth media (for in-house cells) or the specialized medium recommended by the ATCC (for their cells). Cells were used when the monolayers were 60–80% confluent.
**Virus inoculation**

For inoculation, the growth medium was decanted and the monolayers were washed once with the maintenance medium to be used. Then, 0.2 to 0.3 ml of seed virus inoculum (or 0.5 ml of original specimen) was adsorbed to the cells for 1 h at ambient temperature, 1.0 ml of maintenance medium was added, and the tubes were incubated at 36 °C on a roller drum. Cultures were read daily for CPE. The first trial utilized a maintenance medium consisting of Medium 199 with 2% FBS (previously titrated by hemagglutination-inhibition (HI) to be free of bovine parainfluenza 3 SF4 antibodies). Later trials employed Eagle's minimal essential medium (EMEM) supplemented with L-glutamine, penicillin, streptomycin, 0.1% ITS (5 μg/ml insulin/5 μg/ml transferrin/0.005 μg/ml selenous acid, final concentrations; #40350, Collaborative Research, Inc., Lexington, MA), and 1.5 μg/ml of trypsin (#T-0134, Sigma Chemical Co., St. Louis, MO). ITS and trypsin were stored in concentrated form at −70 °C. Final experiments were done under RPMI-1640/glutamine medium (#320-1875PJ, Gibco Labs, Grand Island, NY) with antibiotics and 1.5 μg/ml trypsin.

Viruses were kept cold during inoculation to maintain consistency within the operation and to minimize loss of titer in the more labile viruses (viz., parainfluenza 4A, 4B, mumps). After 7 days, the tubes were scraped and blind-passed at 0.3 ml per tube (3 tubes each). Weekly intervals were chosen for subpassaging because that was consistent with well-known paramyxovirus replication features and with the usable lifespan of many cell cultures. At the end of each passage, one tube was washed with PBS and hemadsorbed with 0.4% guinea pig erythrocytes (0.2 ml of fresh cell suspension in 2.0 ml of PBS, with readings taken after 15 and 45 min at room temperature); the other two were scraped, pooled (including the supernate from the first tube), and subpassaged again. Six serial passages were done, and a portion of each passage was saved at −70 °C for subsequent comparative tests to ascertain virus replication.

**Antigen tests**

In addition to CPE and HAd, hemagglutination (HA), enzyme immunoassays (EIA), and virus infectivity titrations (I.T.) were carried out to measure the extent of viral replication in the cultures. HA titrations were performed according to the standardized microtiter HA procedures, using 0.01 M phosphate-buffered saline (PBS), pH 7.2, as diluent and 0.4% fresh guinea pig or human “O” erythrocytes or 0.5% chicken red blood cells with a 1 h incubation at room temperature [14].

EIA for parainfluenza 1, 2, and 3 was performed in polystyrene flat-bottom microtiter plates (Immulon, Dynatech Laboratories, Alexandria, VA) coated with 75 μl of purified IgG from type-specific horse antisera [13]. EIA for parainfluenza 4A, 4B, and mumps was performed as above except with a biotin-avidin system. The capture IgG was purified from guinea pig antiserum for parainfluenza 4A, ferret antiserum for type 4B, and horse antiserum for mumps. These were added at optimal dilutions in carbonate buffer, incubated overnight, the plates washed, and viral and control antigens added as above. After the 1–1/2 h incubation at 37 °C and washing steps, 75 μl of the corresponding biotinylated guinea pig, ferret, or horse IgG diluted in PBS-GT was added and the plates incubated for 1 h at 37 °C. The remainder of the test was done as previously described [13].

Infectivity titrations were carried out as serial tenfold dilutions of virus culture made in EMEM and inoculated (0.1 ml) in quadruplicate onto appropriate cell monolayers. After a 1 h adsorption at room temperature, 1 ml of maintenance medium was added per tube and the cultures incubated at 36 °C on a roller drum for 14 days. The titers were read by CPE and HAd and calculated by the Reed/Muench method.
Results

Initial evaluation of cell lines

Cell lines were chosen for an initial evaluation on the basis of their morphology, derivation from carcinoma tissue, or derivation from simian kidney tissue (Table 1). Fresh tube cultures of these cells were washed once with maintenance medium (EMEM with 0.1% ITS and 5 μg/ml trypsin), and inoculated with the 6 prototype strains at MOIs of 0.1. The cultures were read daily for CPE. Trypsin at this level was toxic to many of the cell types, so the experiment was repeated with varying doses from 4 to 0.5 μg/ml, final concentration. In general, 1.5 μg/ml was the highest concentration of trypsin tolerated by all the cells. Six sequential passages of the viruses in all of these cells were then made in the presence of 1.5 μg/ml trypsin and evaluated using CPE and HAd as indicators of virus growth, and confluency and viability as indicators of healthy cell controls (Table 2). Many of the cell lines — A-549, G-401, A-498, A-704, CAKI-1, RD, and BSC-1 — grew some of the viruses, but were slow to form monolayers, began sloughing after a few days in culture, or were unreadable for HAd. These were eliminated from further testing. The remaining cells were inoculated with the paramyxoviruses in the presence of the highest level of trypsin they could tolerate. Still, the L132, HEK, Graham-293, LLC-MK2, MA-104, and all 3 Vero lines supported the growth of only some parainfluenzaviruses. Virus replication in these cells was markedly reduced in the absence of trypsin in the medium during the adsorption/entry phase (data not shown). Only the H292 cells supported the growth of all 6 prototype strains.

Table 1. Characteristics of cell lines evaluated

| Cell line | Source                  | Morphology            | Karyology   |
|-----------|-------------------------|-----------------------|-------------|
| L-132     | embryonic lung (human)  | epithelial-like       | heteroploid |
| A-549     | carcinoma lung (human)  | epithelial-like       | heteroploid |
| NCI-H292  | carcinoma lung (human)  | mucoidepidermoid      | polyploid   |
| HEK       | embryonic kidney (human)| fibroblast-like       | diploid     |
| Graham-293| embryonic kidney (human)| Ad5-transformed, epithelioid | aneuploid |
| G-401     | carcinoma kidney (human)| epithelial-like       | diploid     |
| A-498     | carcinoma kidney (human)| epithelial-like       | heteroploid |
| A-704     | carcinoma kidney (human)| epithelial-like       | heteroploid |
| CAKI-1    | carcinoma kidney (human)| epithelial-like       | heteroploid |
| RD        | rhabdomyosarcoma (human)| mixed embryonal       | heteroploid |
| MK        | kidney (rhesus monkey)  | mixed                 | normal      |
| LLC-MK2   | kidney (rhesus monkey)  | epithelial-like       | heteroploid |
| BSC-1     | kidney (Afr. green monkey)| epithelial-like      | heteroploid |
| MA-104    | kidney (Afr. green monkey)| epithelial-like     | heteroploid |
| Vero-CCL81(ATCC) | kidney (Afr. green monkey) | fibroblast-like     | heteroploid |
| Vero-76 (ATCC) | kidney (Afr. green monkey) | fibroblast-like     | heteroploid |
| Vero-76/E6 (CDC) | kidney (Afr. green monkey) | fibroblast-like     | heteroploid |
Table 2. Initial evaluation of cells

|       | MK  | LLC-MK2 | Vero 81 | Vero 76 | Vero E6 | L-132 | A-549 | NCI-H292 | HEK | G-401 | A-498 | A-704 | Caki-1 | RD |
|-------|-----|---------|---------|---------|---------|-------|-------|----------|-----|-------|-------|-------|--------|-----|
| Para. 1 | +   | -       | -       | +       | +       | -     | -     | +        | -   | -     | -     | -     | -      | -   |
| Para. 2 | +   | +       | +       | +       | +       | +     | +     | -        | -   | -     | +     | +     | +      | +   |
| Para. 3 | +   | +       | +       | +       | +       | +     | +     | +        | +   | -     | +     | +     | +      | +   |
| Para. 4 A | +   | -       | -       | -       | -       | -     | -     | +        | nd | nd    | nd    | -     | nd     | nd |
| Para. 4 B | -   | -       | -       | -       | -       | -     | -     | +        | nd | nd    | nd    | nd    | nd     | -   |
| Mumps  | +   | +       | +       | +       | +       | +     | +     | +        | nd | nd    | nd    | +     | nd     | +   |
| Cell controls | OK | OK | OK | OK | OK | OK | OK | OK | not | not | not | not | not | not |

+ Growth after 6 passages
- No growth
nd Not done
Comparison of prototype strains in MK and H292 cells

In the next trial, we compared H292 cells directly with MK cells in 6 sequential passages of the prototype strains (Table 3). Six passages were done to be sure the inoculum was diluted out and we could observe increases in antigen titer that would reflect viral replication. Each antigen test was performed with all passages in both cells in parallel to ensure an accurate comparison. Values for passage #6 are shown in Table 3.

The H292 cells were thus shown to: (a) propagate all 6 paramyxoviruses to higher titers than other continuous lines and to titers equivalent to those obtained in MK cells; (b) be maintainable in the laboratory without special measures; (c) produce cell monolayers that are easy to read for CPE; and (d) have a cell morphology that is distinguishable from erythrocytes in HAd tests.

Comparison of wild strains in MK and H292 cells

Next, we tested the ability of H292 cells to support the growth of diverse paramyxovirus isolates obtained in MK cells over a 30-year period and geographical spread. The viruses were subpassaged 6 times in H292 and MK cells in parallel and the passages were assayed by CPE, HAd, HA titrations, and EIA. The results are indicated as the number of isolates recoverable in each cell type at the end of 6 passages (Table 4). The strains of parainfluenza 1, 2, and 3 were recovered in the first or second passage in both cell systems, while the strains of parainfluenza 4 and mumps required 3 to 4 passages, as expected from the general lability of these viruses. All viruses were recovered except for 1 strain of mumps in MK cells and 2 in H292 cells. Thus, the H292 cells are clearly susceptible to a broad range of strains.

A review of the diagnostic features of the different serotypes in H292 cells revealed trends in the type of CPE, pattern of HAd, and titer of HA, that were

| Virus | Strain | CPE   | HAd   | HA    | EIA    | I.T. (log10) |
|-------|--------|-------|-------|-------|--------|-------------|
|       |        | MK    | MK    | MK    | MK     | MK          | MK          | H292      |
|       |        | 3+    | +     | +     | 64     | 0.885      | 1.020       |           |
|       | Greer  | 4+    | +     | +     | 2      | 1.485      | 1.974       |           |
|       | C-243  | 1+    | 4+    | +     | 64     | 1.522      | 1.846       |           |
|       | M-25   | 2+    | 3+    | +     | 2      | 0.660      | 0.497       | 4.0       |
|       | Mumps  | 1+    | 3+    | +     | 2      | 0.033      | 0.345       | <1.0      |

* Indicators of growth after 6 passages: cytopathology (CPE), hemadsorption (HAd), and hemagglutination (HA) titer are as described in Materials and methods. EIA value is defined as the absorbance at 450 nm of undiluted antigen (culture supernatant) in polyclonal EIA tests (types 1-3) or biotin-avidin EIA tests (type 4 and mumps). Infectivity titer is the log10 TCID50 per ml at 7 days.
Table 4. Recovery of stored paramyxovirus isolates in MK and NCI-H292 cells

| Virus   | No. of isolates | MK       | H292     |
|---------|-----------------|----------|----------|
| Para. 1 | 18              | 18       | 18       |
| Para. 2 | 19              | 19       | 19       |
| Para. 3 | 15              | 15       | 15       |
| Para. 4A| 8               | 8        | 8        |
| Para. 4B| 4               | 4        | 4        |
| Mumps   | 18              | 17       | 16       |
| Total   | 82              | 81 (98.8%)| 80 (97.6%)|

similar to those seen in MK cells. CPE ranged from inapparent to degenerative to syncytial, depending on the amount of virus present, the length of culture, and the virus type. Certain types produced a predominantly degenerative appearance with some aggregation (e.g., parainfluenza 1); others produced a somewhat syncytial pattern (e.g., parainfluenza 2, 4A). Parainfluenza 3 produced the most recognizable syncytial CPE, and mumps was distinct in the amount of fusion produced. Still, there was sufficient overlap in CPE descriptions between virus types and among strains of the same virus that CPE was not a reliable predictor of virus type. CPE description was further confused by the relatively low degree of CPE observed, ranging from 1+ (= 25% of the cell monolayer visibly affected) in mumps-infected cultures to 4+ (= 100% of cells affected) in parainfluenza 3-infected cultures. Hemadsorption in H292 cell cultures also was similar to that seen in MK cultures; parainfluenza 1 and 2, and some cultures of parainfluenza 3 and mumps gave uniform HAd, with the erythrocytes evenly and solidly spread over the cell monolayer; but cultures of parainfluenza 4 and some of parainfluenza 3 and mumps gave patchy HAd patterns, usually at the edges of the monolayers. HA tests with guinea pig cells showed the same grouping of HA titers in H292 cultures as in MK cultures: parainfluenza 1, 2, and 3 strains typically gave the highest titers, ranging from 1:8 to 1:512; parainfluenza 4 strains gave the lowest titers (1:1−1:16); and mumps strains gave intermediate titers of 1:4−1:64. FA tests on virus-infected H292 cells also exhibited various staining patterns among the strains studied, as in MK cultures, but the patterns were not consistent with particular virus types. Therefore, the growth parameters observed (variations in CPE, HAd, HA titer, and FA staining) may be suggestive of virus type but are not reliable characteristics of a serotype.

Primary isolation in H292 cells

The final phase of the study was a prospective comparison of H292 cells with MK cells with incoming clinical specimens (Table 5). Specimens were processed by our routine procedures [13], inoculated into H292 cells and MK cells,
Table 5. Primary isolation of human paramyxoviruses in NCI-H292 cells

| Virus      | No. of viruses recovered in | Mean no. days of culture |
|------------|----------------------------|--------------------------|
|            | MK | H292 | MK | H292 |
| Para. 1    | 7  | 7    | 7  | 11   |
| Para. 3    | 13 | 13   | 5  | 7    |
| Mumps      | 10 | 11   | 12 | 10   |

Subpassaged and tested in both cells in parallel. For parainfluenza 1 and 3, identical results were obtained in both cell lines, although the viruses were detected in MK cells in slightly less time than in H292 cells. For mumps virus, the H292 cells gave clearer CPE and in less time than in MK cells. No strains of parainfluenza 2, 4A, or 4B were recovered from the community during this period of testing.

Production of high-titered stocks in H292 cells

Stocks of the paramyxoviruses were then prepared in H292 cells and evaluated by the parameters above and by electron microscopy to ascertain their usefulness as seed viruses (Table 6). The viruses were prepared as pools of infected cultures.

Table 6. Growth parameters of human paramyxovirus seed stocks prepared in NCI-H292 cells

| Virus  | Strain | CPE degree/type | HAd\(^a\) chick g. pig | HA titer\(^b\) | EIA titer\(^c\) | Infectivity\(^d\) |
|--------|--------|-----------------|-------------------------|----------------|----------------|------------------|
| Para. 1| C-35   | 2+/degen./aggreg.| u                        | 32             | 512            | 512              |
| Para. 2| Greer  | 3+/variable-syncyt.| u                      | 32             | 256            | 64               |
| Para. 3| C-243  | 4+/degen./syncyt. | u/p                     | 1              | 256            | 128              |
| Para. 4A| M-25  | 1+/variable-syncyt.| p                      | 1              | 8              | 1                |
| Para. 4B| 19503 | 1+/variable/aggreg. | p/p                   | 1               | 8              | 1                |
| Mumps  | Enders | 1+/fusion/sync./degen. | u/p                  | 16             | 64             | 16               |

\(^{a}\) Uniform, p patchy hemadsorption at 30 min, 23°C

\(^{b}\) HA titer is the reciprocal of the highest dilution of antigen (culture supernatant) exhibiting complete agglutination with chicken, guinea pig, and human "O" erythrocytes in 1 h at ambient temperature

\(^{c}\) EIA titers are noted as the \(\log_{10}\) of the highest dilution of antigen that exhibits a P/N (positive/negative) value of \(\geq 3.0\)

\(^{d}\) Infectivity titers are noted as the \(\log_{10}\) TCID\(_{50}\) per ml after 14 days of incubation in roller culture at 36°C
and harvested by one freeze-thaw cycle after 4 to 7 days of incubation. All of
the new seed viruses were high-titered by the activity tests, consisted of typical
intact paramyxovirus by electron microscopy, and were devoid of bacterial,
fungal, and mycoplasmal contamination by extensive culturing in appropriate
media. EIA, HI, and breakthrough neutralization tests with paramyxovirus
antisera showed no cross-contamination within the group or with other viruses.
The data in Table 6 were obtained by replicate tests on vials of virus after a
total of two freeze-thaw cycles; HA and EIA titers were consistently higher on
samples after multiple freeze-thaw cycles or after storage up to 8 weeks at 4 °C.
These data show that virus seeds prepared in NCI-H292 cells are equal in virus
titer to those formerly prepared in MK cells; the other antigen tests gave equally
parallel results, although the MK data are not shown here.

Discussion

The parameters we employed in selecting a new cell line were based on utility
in clinical virology laboratories. (a) The cell stock had to be readily maintainable,
so that adequate reserves could be frozen back for future use. (b) Cell stability
during the incubation period was important because paramyxoviruses are fairly
slow-growing, requiring 5 to 7 days of culture to become evident. (c) The virus-
induced CPE ideally should be obvious, but more importantly, the host cells
and their nuclei had to be distinct from the erythrocytes used in the HAd test
so that the presence of virus could be detected with the same reliability it is in
MK cells. (d) Finally, the cells had to be susceptible to infection by all serotypes
of human paramyxoviruses.

In selecting the cell types to be evaluated in this study, we considered the
natural pathogenesis of paramyxovirus infection in man, namely, that the viruses
first become established in the mucous membranes of the nose and throat [9].
Therefore, epithelial cells from these sites would be likely candidates for sensitive
cells. In severe infections, the lower respiratory tract is involved, and therefore
human lung cells might be used. Because both human and rhesus kidney cells
are sensitive to the paramyxoviruses [8–10], cell lines derived from primary or
tumor cells from these sites were candidates. We used only selected cells derived
from African green (vervet) monkeys because primary African green kidney
cells are known to be less sensitive than primary rhesus kidney cells for isolating
paramyxoviruses [5, 9]. Consideration was also given to previous studies which
had shown some promise with Vero cells [16, 17, 23, 26, 27, 33], LLC-MK2
[12, 21], A-549 cells [30, 34], and others [29, 31, 32].

Many cells were not adequate because they had poor growth characteristics
and additionally did not support the growth of all 6 human paramyxoviruses.
There was no consistent pattern of lack of virus growth, except that parainfluen-
za 1, 4A, and 4B were the most difficult to grow in all cells (even MK), and
parainfluenza 3 and mumps were the least difficult, as is known from previous
experience [9, 12, 21]. Some of the cells we evaluated (such as BSC-1) may
have been subjected to such high passage levels that they had become less differentiated and had possibly lost the specific receptor sites needed for paramyxovirus attachment. This may account for the failure in our hands to grow parainfluenza 4 in Vero cells, in contrast to good success in an earlier study [26].

Of the cells we evaluated, only NCI-H292 cells (ATCC # CRL-1848) supported the growth of all the human paramyxoviruses. The H292 cells are a continuous line of mucoepidermoid carcinoma cells derived from human lung in 1985 [4, 6]. They possess many of the characteristics we originally considered because they are continuous cells of human respiratory-tract origin. H292 is a relatively unknown cell line, but has been used previously for hepatitis B antigen transfection [35].

Although H292 cells were initially grown in medium containing insulin, transferrin, and selenium [6], we found these unnecessary for either cell maintenance or virus replication in our studies. Instead, RPMI-1640 was satisfactory as both growth and maintenance medium. Trypsin was considered an important additive to the medium because earlier studies had reported significantly enhanced replication for a number of viruses after treatment with, or in the presence of, trypsin [1–3, 12, 15, 17, 20, 28]. Although MK cells do not require trypsin to support the growth of orthomyxoviruses or paramyxoviruses [12, 21, 25], influenza virus is activated by trypsin in other cells such as chick embryo cells [20, 22], MDCK [12, 22, 24], and Vero [17]; and some paramyxoviruses are enhanced by trypsin in LLC-MK2 [12, 21], Vero [17, 26, 27], and HEP2 [29]. The level of trypsin used varies greatly between viruses, cell types, and laboratory procedures. In our studies, we found trypsin to be essential for the replication of paramyxoviruses in H292 cells under either EMEM or RPMI maintenance media.

The final protocol for culturing the human paramyxoviruses in H292 cells consists of growing the cells to ~70%-confluent monolayer under RPMI-1640 medium with 10% FCS, decanting the growth medium, rinsing the cells once with 1 ml of maintenance medium (RPMI-1640 with 1.5 μg/ml trypsin), inoculating with 0.3 to 0.5 ml of specimen, adsorbing for 1 h at ambient temperature, adding 1 ml of the maintenance medium, and incubating at 36 °C on a rocker or roller drum. The cultures are then followed for CPE at 2, 4, and 7 days, subpassaged to provide for maximum virus recovery (we prefer 3 subpasses at weekly intervals), and hemadsorbed with guinea pig erythrocytes as usual.

In these studies, we have demonstrated that H292 cells are a promising substitute for primary rhesus monkey kidney cells for the isolation and propagation of the human paramyxoviruses. Initial clinical studies also suggest that this cell line supports primary isolation of a broad range of other viruses, including adenoviruses, enteroviruses, rhinoviruses, herpesviruses, and some strains of influenza viruses and respiratory syncytial virus. Detailed studies of the virus spectrum of H292 cells are in progress and will be reported separately.
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