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Short communication

Comparison of HeLa-I, HEp-2 and NCI-H292 cell lines for the isolation of human respiratory syncytial virus (HRSV)

Ana Priscila Perini [a], Maria Luisa Barbosa [a,c], Viviane Fongaro Botosso [a,d], Claudia Trigo Pedroso de Moraes [a], Alfredo E. Gillio [b], Noeli Hens [b], Klaus E. Stewien [a], Edison L. Durigon [a,*]

[a] *Universidade de São Paulo, Instituto de Ciências Biomédicas, São Paulo, Brazil*
[b] *Universidade de São Paulo, Hospital Universitário, São Paulo, Brazil*
[c] *Instituto Adolfo Lutz, São Paulo, Brazil*
[d] *Instituto Butantan, São Paulo, Brazil*

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Abstract

Generally, laboratory diagnosis of viral respiratory infections utilizes virus isolation in cell culture and immunofluorescence assays. In this study, three cell lines (HEp-2, NCI-H292 and HeLa-I) were used for HRSV isolation of strains obtained from patients admitted at HU-USP with respiratory tract disease. HRSV was isolated in 46% (37) of 80 specimens inoculated in HeLa-I, 48% (39) in HEp-2, and 36.3% (29) in NCI-H292. Immunofluorescence was considered the gold standard and yielded 53% positive (43). The results from both methods combined had better sensitivity (73.2%) compared to either method alone. Comparing results between the cell lines with HEp-2 cells as the benchmark, the greatest sensitivity (72.2%) was observed in HeLa-I. This data shows that HeLa-I is adequate for HRSV isolation, giving results similar to the HEp-2 cells. The combined use of the HEp-2, HeLa-I and NCI-H292 cells improve the detection of HRSV.

Keywords: HRSV; Cell culture; Immunofluorescence assay

Respiratory syncytial virus (RSV) was described for the first time in 1956 in chimpanzees with symptoms of respiratory disease (Morris et al., 1956). Later, this agent was detected in humans (Chanock and Finberg, 1957a,b). Different studies show the importance of this virus in young children, elder and immunocompromised patients (Glezen and Denny, 1973; Martin et al., 1978; Falsey et al., 1990; Falsey, 1998; Hertz et al., 1989). Currently, the use of immunofluorescence or enzyme immunoassay for direct detection of HRSV antigen provides rapid and low cost diagnosis in the laboratory when compared with viral isolation in cell culture and polymerase chain reaction. The use of the shell vial technique has become an important adjunct to conventional tube culture methods by improving the rate of detection and overall sensitivity with several viruses.

HRSV consists of a nucleocapsid contained within a lipid envelope that is derived from the host plasma membrane. Spikes of G and F glycoproteins, which are responsible for virus attachment to the cell as well as for membrane fusion, are organized in the enveloped surface. The interaction between the viral G protein and glycosaminoglycans present at the cell's surface was shown (Krusat and Streckert, 1997; Hallak et al., 2000a). However, it is not clear if this is only an initial step to the binding of virus to a high affinity receptor (Martínez and Melero, 2000; Hallak et al., 2000b). Behera et al. (2001) described the interaction between ICAM-I molecule (intra-cellular adhesion molecule I) with viral F protein, facilitating the entrance of the virus and infection of HEp-2 cells.

In this study, HRSV isolation was studied in three cell lines: HEp-2, a classical cell line used for HRSV propagation; NCI-H292, a continuous human lung muco-epidermoid cell line that is an excellent substitute for primary rhesus monkey kidney cells for the isolation and propagation of all human paramyxoviruses, and also presents ICAM-I expression (Castells et al.,

* Corresponding author at: Department of Microbiology, Institute of Biomedical Science, University of São Paulo, Brazil. Tel.: +55 11 30917293; fax: +55 11 30917354.
E-mail address: eldurigo@usp.br (E.L. Durigon).

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1990; Hierholzer et al., 1993; Molock and Lillehoj, 2006); HeLa-I cells (Arruda et al., 1996) that expressed ICAM-I at double the level of HeLa cells, strain Ohio and 52 times the HeLa cells level, strain 299, a non-ICAM-I-expressing strain. Indirect immunofluorescence assay was considered the gold standard technique for comparison.

From January to October 2000, nasal swabs and nasopharyngeal aspirates were obtained from 80 children under 1-year-old admitted to the intensive care unit (ICU), and pediatric wards of the University Hospital at Sao Paulo University (HU-USP) with upper and lower respiratory tract infection. This study was approved by the Medical Ethic Commission of the University Hospital and Biomedical Sciences Institute from São Paulo University (ICB-USP). Samples were taken by inserting a dry swab into one nostril. The swab was allowed to remain in the area for 10–30 s, rotated and withdrawn, and placed into 3.5 mL of phosphate-buffered saline (PBS) of 0.01 M pH 7.2. A sample was then obtained from the other nostril by nasopharyngeal aspiration. Specimens were held at 4 °C until transported to the laboratory.

The nasopharyngeal aspirate and the swabs were mixed, and treated with 1000 U/mL of penicillin (Gibco BRL), 1000 μg/mL of streptomycin (Gibco BRL), and 50 U/mL of Nystatin (Gibco BRL). Direct smears were prepared by centrifuging the specimen at 450 g for 10 min, and applying part of the resulting pellet onto a slide. Once dried, the slides were fixed in acetone P.A. (Merck) for 10 min, and air-dried before staining. Cell spots were stained with 10 μL of specific MAbs: 8661 (anti-influenza A); 8251 (anti-influenza B); 834-2 (anti-parainfluenza 1); 844-3 (anti-parainfluenza 2); 855-1, 855-2, 855-3 (anti-parainfluenza 3); 805 (anti-adenovirus); 858-4 (anti-respiratory syncytial virus). 92-11c (anti-HRSV A) and 102-10b (anti HRSV B) MAbs were used for grouped HRSV positive specimens (Anderson et al., 1985, 1991). After 60 min of incubation at 37 °C and 10 min of washing in PBS, 10 μL of anti-mouse IgG conjugate with fluorescent isothiocyanate was added on the cell spots. The slides were incubated again for 60 min at 37 °C. Following a 10 min wash in PBS slides were mounted in glycerol and examined for the presence of fluorescent-labeled cells.

HEp-2 and HeLa-I cells were grown in Eagle's minimal essential medium supplemented with 10% fetal bovine serum (FBS), and NCI-H292 cells in RPMI-1640 with FBS, at 37 °C in CO_2 incubator. They were subcultured at weekly intervals with a trypsin-EDTA solution (Trypsin and 0.2% EDTA). For virus isolation, the cell lines were lightly seeded in 24-well plates and used within 1–2 days of seeding when the cell layer was still subconfluent.

Specimens were inoculated in duplicate into each cell line, the virus inoculum being added after removal of culture medium, and then allowed to adsorb for 1 h at 37 °C in CO_2 incubator. Fresh maintenance medium (MEM with 2% FBS) was added to each well, and the plates were incubated at 37 °C in a CO_2 incubator. As control, two wells were not inoculated. The plates were examined daily from 10 to 20 days for cytopathic effect (CPE). Indirect immunofluorescence of positive samples was performed with specific MAbs described previously. Cultures, which did not show CPE 7 days post-infection were blind passaged and then stained with specific MAbs. HRSV was identified in cell culture by syncytium development on average at day 6 in HeLa-I and HEp-2, and day 9 in NCI-H292 (Fig. 1). The presence of virus by immunofluorescence was determined by observation of granulate fluorescence in the membrane and cytoplasm with an UV microscope at magnifications of 100× and 400×.

Fifty-eight of the 80 samples evaluated were positive for HRSV by at least one of two methods employed, and 31 were positive in both. By IFA, 43 (53.75%) were positive for HRSV (31 HRSV-A; 5 HRSV-B; 7 HRSV not grouped), and two samples were positive for Flu-A, and two for adenovirus. Four samples were considered inconclusive, because of inadequate cell number (less than 20 cells per area), and/or the presence of excess of mucus complicating the interpretation Two samples were not submitted for immunofluorescence (Table 1), and were excluded from IFA comparison.

Of the 31 HRSV type A identified by IFA 21 (68%) were isolated in HeLa-I cells, 22 (71%) in HEp-2, and 16 (52%) in NCI-H292; 5 HRSV type B detected by IFA were isolated in HEp-2 and NCI-H292, and four of them (80%) in HeLa-I. Three of the seven samples positive by IFA but not typed were isolated in all cell lines; however, they did not react with group specific antibodies. Therefore, six positive samples by IFA for HRSV were negative in cell cultures whereas 13 negative samples and 2 inconclusive samples by IFA were isolated in at least one of the cell lines. Thus, HRSV detection was raised from 43 positive by IFA alone to 58 positive samples by IFA and isolation in cell culture, an increase of 19.2% in detection rates (Table 1). Discrepancies in results of IFA and culture showed the need for complementary use of culture and alternative methods for HRSV detection (Ahluwalia et al., 1987; Dickison and Tilton, 1987; Hughes et al., 1988; Brumback and Wade, 1996).

When tissue culture isolation results were compared with IFA results, the HEp-2 cells show the best sensitivity of 73.2%. For this analysis we excluded two samples are not examined by IFA, three specimens with microbial contamination and four samples considered are inconclusive by IFA. When the three cell lines were compared and HEp-2 cells were used as the benchmark, HeLa-I showed 66.7% sensitivity. The concordance analysis showed $\kappa = 0.43$ between HEp-2 × NCI-H292 and HEp-2 × HeLa-I cells, considered a moderate concordance, and a regular concordance ($\kappa = 0.34$) between HeLa-I and NCI-H292 (three samples with contamination were excluded from concordance analysis). NCI-H292 isolated less HRSV (29/77) than the other cells.

These results showed there are no differences between HeLa-I and HEp-2 for HRSV isolation but evidence of interaction between ICAM-I and HRSV F protein in virus entry has been documented (Behera et al., 2001). However, the precise role of HRSV cell receptor as a determinant of tissue tropism remains unclear and needs further investigation.

Arens et al. (1986) report that the best results for HRSV isolation were obtained with the use of more than one cell line. The authors showed an increase from 71 to 76% on HRSV isolation rates with the use of HEp-2 cells, primary culture from rhesus monkey's kidney (RhMK), and fibroblast from human lung (WI-38 or MRC-5). In the present study, the use of dif-