

## Enhanced Isolation of Respiratory Syncytial Virus in Cell Culture

MAX Q. ARENS,<sup>1\*</sup> ELLA M. SWIERKOSZ,<sup>1,2</sup> ROSE RITA SCHMIDT,<sup>1,2</sup> THOMAS ARMSTRONG,<sup>2</sup> AND  
KAREN A. RIVETNA<sup>2</sup>

*Department of Pediatrics and Adolescent Medicine, St. Louis University School of Medicine,<sup>1</sup> and Diagnostic Virology Laboratory, Cardinal Glennon Children's Hospital,<sup>2</sup> St. Louis, Missouri 63104*

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**During two winter seasons, we found that the combination of WI-38 or MRC-5 human lung fibroblasts plus primary rhesus monkey kidney (RhMK) and HEp-2 cell cultures yielded maximal isolation of respiratory syncytial virus. Cytopathic effects (CPE) developed earliest in RhMK cells and slowest in the human fibroblast lines. In RhMK cells, 50% of ultimately positive cultures showed CPE in 5 days, and 90% of positive cultures showed CPE within 7 days during both respiratory syncytial virus seasons.**

Acute respiratory disease caused by respiratory syncytial virus (RSV) is a particularly debilitating infection in infants and young children. The usual manifestations are upper respiratory disease with rhinitis and fever, which often progress to bronchiolitis, pneumonia, or both in primary infections, especially in infants less than 6 months of age (2).

Early and accurate diagnosis of RSV infections may reduce the use of antibiotic therapy and limit the spread of nosocomial infections. Furthermore, the anticipated availability of RSV-specific therapeutic agents makes expeditious diagnosis essential for patient management (5, 8).

In our institution, RSV infections occur in annual winter outbreaks with 50 to 150 cases each season. During the winters of 1982 to 1983 and 1983 to 1984, we sought to determine the most rapid and sensitive methods of RSV diagnosis. Both virus isolation with various cell types and detection of RSV-infected cells in nasopharyngeal specimens by immunofluorescence (IF) were used. In this paper, we describe our use of various cell cultures for early and efficient detection of RSV.

Specimens from infants and children with acute respiratory disease were cultured for viral studies at their physicians' request. Specimens were taken from December 1982 to July 1983 (group 1) and from August 1983 to March 1984 (group 2).

Nasopharyngeal swabs were placed in vials of viral transport medium (veal infusion broth, 0.5% gelatin, gentamicin [100 µg/ml], mycostatin [100 U/ml], penicillin G [200 U/ml], amphotericin B [5 µg/ml], 0.00125% phenol red) (2.5 ml per vial). Specimens from hospitalized patients usually arrived in the virology laboratory within 4 h after collection. Specimens received from outside hospitals were kept at 4°C until transport to the laboratory.

Cell culture tubes of human lung fibroblasts (WI-38 or MRC-5) and primary rhesus monkey kidney (RhMK) cells containing simian virus 5 (SV5) and SV40 antisera were obtained weekly from Flow Laboratories, McLean, Va. Lung fibroblasts were maintained on roller drums at 36°C. RhMK cells were maintained on stationary racks at 33°C.

HEp-2 cells were purchased from Flow Laboratories as suspension cultures at passages 369 to 383. Tubes were seeded twice a week at 37°C in medium with 2 or 10% fetal bovine serum (FBS) either directly from the suspension or from cells trypsinized from flasks which had been made from

the suspension. When the cells reached 75% confluence, the tubes were fed with complete medium with 2% FBS and maintained at 33°C. Following inoculation, all HEp-2 cells were maintained at 33°C, since at higher temperatures they grow rapidly to confluence then round up and slough from the tubes.

Human embryonic kidney (HEK) cell culture tubes were obtained from Whittaker M. A. Bioproducts, Walkersville, Md. and maintained in complete medium with 10% FBS on stationary racks at 33°C. The medium in all cell culture tubes was changed the day after arrival. All cells were fed with minimum essential medium with 0.4% sodium bicarbonate, 100 U of potassium penicillin G per ml, 25 µg of gentamicin sulfate per ml (complete medium) containing heat-inactivated FBS as described below. Prior to inoculation, all cell culture tubes were fed with inoculation medium and then fed again at 7 days postinoculation (p.i.). Inoculation medium contained 10% FBS (WI-38, MRC-5, and HEK cells), 2% FBS (HEp-2 cells), 2% FBS and 0.1% each SV5 and SV40 antisera (RhMK cells at 37°C), or no FBS but with 0.1% each SV5 and SV40 antisera and 2 µg of trypsin per ml (RhMK cells at 33°C).

Most specimens were set up on both WI-38 and MRC-5 cell lines, and the earliest day that either one showed RSV cytopathic effect (CPE) was taken as the day of reportable CPE for the pair. Owing to varying availability of cells, 94 of the 95 culture-positive specimens in group 1 and 97 of the 122 culture-positive specimens in group 2 were set up on all cell lines.

Nasopharyngeal specimens were mixed by Vortex, and 0.2 ml was inoculated into cell culture tubes. One tube each of HEK, WI-38, MRC-5, and RhMK cells was incubated at 36°C on a roller drum. In addition, one tube of WI-38 (for

TABLE 1. Comparison of cell culture types for isolation of respiratory syncytial virus (1982 to 1983; group 1)—rate of development of reportable RSV CPE in various cell types in 94 positive specimens

Cell culture type	No. of specimens positive (%)	Earliest day of positivity	Day ≥50% of cultures positive	Day ≥90% of cultures positive
HEp-2	69 (73)	3	6	10
WI-38 or MRC-5	71 (76)	3	9	14
RhMK	67 (71)	3	5	7

\* Corresponding author.

TABLE 2. Comparison of cell culture groups for isolation of RSV (group 1)

Cell culture group	No. positive (total = 94)	% Positive
WI-38 or MRC-5 with HEp-2 and RhMK	94	100
WI-38 or MRC-5 with RhMK	90	96
WI-38 or MRC-5 with HEp-2	91	97
HEp-2 with RhMK	87	93

detection of rhinovirus) and two tubes each of RhMK (for detection of influenza and parainfluenza by hemadsorption) and HEp-2 cells were incubated at 33°C on a roller drum. Cell cultures were observed daily (except Sunday) for 7 days, and every third or fourth day thereafter for 14 days. HEp-2 cells were read daily (except Sunday).

RSV was identified by the formation of large syncytial cells which failed to hemadsorb quinea pig erythrocytes. Identification by CPE correlated well with IF results on the original specimens. Of 420 specimens, 95% of the culture-positive specimens were also IF positive, and 83% of the culture-negative specimens were also IF negative. Questionable CPE was confirmed by IF on the cell cultures. IF was performed essentially as previously described (7). Cells eluted from nasopharyngeal swabs or from culture tubes were washed with phosphate-buffered saline (PBS), resuspended in PBS, spotted onto microscope slides, air dried, and then fixed in acetone at -20°C. These cells were stained with a commercial polyclonal direct conjugate (Flow Laboratories) or indirect reagents (Burroughs Wellcome Co., Research Triangle Park, N.C.) and observed by fluorescence microscopy for specific staining. Foamy simian viruses were not evident in RhMK cell cultures considered positive for RSV. In virtually every case, culture tubes at 36°C showed RSV CPE before or at the same time as tubes kept at 33°C.

For group 1 isolates, the most sensitive cell lines were human fibroblasts (WI-38 and MRC-5), followed by RhMK and HEp-2 (Table 1). Whereas the earliest day p.i. to development of RSV CPE was similar for all three cell lines, the progression of CPE appeared to be most rapid in RhMK and HEp-2 cells. By days 5 and 6 p.i., 50% of the ultimately positive cultures were positive in RhMK and HEp-2 cells, respectively. In contrast, it was not until day 9 p.i. that 50% of the WI-38 or MRC-5 cultures were positive. Ninety percent or more of positive cultures were detected by day 7 p.i. in RhMK, day 10 in HEp-2, and day 14 in WI-38 or MRC-5 cells.

Combinations of cell cultures were more efficient than any individual cell type for isolation of RSV. The use of a single cell type would have failed to detect 24 to 29% of isolates (Table 1), whereas maximal isolation was achieved with the WI-38 or MRC-5 plus HEp-2-RhMK cell combination. Lung

TABLE 4. Comparison of cell culture groups for isolation of RSV (group 2)

Cell culture group	No. positive (total = 97) <sup>a</sup>	% Positive
WI-38 or MRC-5 with HEp-2 and RhMK	96	99
WI-38 or MRC-5 with RhMK	91	94
WI-38 or MRC-5 with HEp-2	88	91
HEp-2 with RhMK	92	95

<sup>a</sup> One specimen developed CPE only in HEK cells, which rarely grow RSV but are routinely included in respiratory setups for detection of adenovirus.

fibroblasts in combination with HEp-2 or RhMK were capable of growing 97 and 96% of potential isolates in group 1, respectively, and the combination of HEp-2 and RhMK cells detected 93% of group 1 isolates (Table 2).

In group 2 isolates, WI-38 and MRC-5 cells were the least sensitive in detection of RSV (Table 3) and, if used alone, would have missed 35% of the total isolates. In this group, RhMK cells were able to support the growth of 88% of the isolates, and the development of CPE in ≥90% of ultimately positive cultures was earliest in this cell line. HEp-2 also showed early expression of CPE, with 90% of total isolates becoming positive within 8 days. Maximal isolation was obtained with the WI-38 or MRC-5 plus HEp-2-RhMK combination (Table 4). The HEp-2-RhMK combination was the second most efficient cell group (95%), in contrast to group 1 specimens, in which the HEp-2-RhMK group was least efficient (91%).

The differences in sensitivity of the various cell lines from one year to the next cannot be predicted. In group 1, fibroblasts were most sensitive, having detected 76% of the isolates, but in group 2 they were the least sensitive and grew only 65% of the isolates. RSV isolations in RhMK cells were also considerably different between the two seasons. Whether this difference was due to different passages or conditions of cell lines or both or to the changing prevalence of different strains of virus from one year to the next is unknown. It does, however, point out the need to use as many cell lines as is economically reasonable in an effort to maintain a high RSV detection level. The use of HEp-2 cells alone, as others have suggested (1, 3, 4, 6), would have missed about 25% of the total isolates in this study. Moreover, the use of a number of cell culture types allowed for overall earlier detection of RSV, which is of prime importance for limiting nosocomial spread and when antiviral therapy is contemplated.

Our data indicate that the use of lung fibroblasts, primary RhMK cells, and HEp-2 cells in combination contributes to enhanced isolation of RSV. Our data indicate that combinations of any two of these cell lines would be effective in isolation of 94 to 95% of the isolates that all three could

TABLE 3. Comparison of cell culture types for isolation of RSV (1983 to 1984; group 2)—rate of development of reportable RSV CPE in various cell types in 97 positive specimens

Cell culture type	No. (%) of specimens positive (total = 96) <sup>a</sup>	Earliest day of positivity	Day ≥50% of cultures positive	Day ≥90% of cultures positive
HEp-2	74 (77)	3	5	8
WI-38 or MRC-5	62 (65)	3	10	14
RhMK	84 (88)	3	5	7

<sup>a</sup> One specimen developed CPE only in HEK cells, which rarely grow RSV but are routinely included in respiratory setups for detection of adenovirus.

detect. These cell types are commonly available in many diagnostic virology laboratories for isolation of a variety of viruses, and their use should be expanded to include specimens for possible RSV isolation.

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