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Rapid Detection of Respiratory Viruses by Shell Vial Assay Using Simultaneous Culture of HEp-2, LLC-MK2, and MDCK Cells in a Single Vial

JOSÉ M. NAVARRO-MARÍ,* SARA SANBONMATSU-GÁMEZ, MERCEDES PÉREZ-RUIZ, AND MANUEL DE LA ROSA-FRAILE

Servicio de Microbiología, Hospital Universitario Virgen de las Nieves, 18014 Granada, Spain

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A shell vial assay with simultaneous culture of HEp-2, LLC-MK2, and MDCK cell lines in a single tube (CoHLM SV assay) was compared with traditional tube culture (TC) for the detection of the main respiratory viruses in 358 nasal wash specimens. A total of 170 strains were isolated from 168 virus-positive samples. A total of 94.1% of the strains (160 strains; 128 respiratory syncytial viruses and 32 other viruses) were detected by the CoHLM SV assay in 48 h, whereas 98.2% of the strains (167 strains; 132 respiratory syncytial viruses and 35 other viruses) were detected by TC in a mean time of 6 days. The CoHLM SV assay may be useful for the rapid detection of respiratory viruses.

Centrifugation-enhanced shell vial (SV) culture is becoming one of the most frequent techniques used in clinical virology laboratories since it reduces the turnaround time (4, 7, 9, 10, 12).

The SV assay has been shown to have a good correlation with traditional tube culture (TC) for the detection of the main respiratory viruses (RVs) implicated in acute pathology: respiratory syncytial virus (RSV), influenza A virus (FLU A), influenza B virus (FLU B), parainfluenza viruses 1, 2, and 3 (PARA-1, PARA-2, and PARA-3, respectively), and adenovirus (AD) (9, 13, 14). However, no cell line is universally sensitive to all RVs; thus, the inoculation of each specimen must be carried out in numerous vials containing different cell lines. In this study we propose a simplification of the assay by performance of simultaneous culture of three cell lines (HEp-2, LLC-MK2, and MDCK cells [CoHLM]); in the same vial, thus permitting growth of the main RVs.

For this purpose, the HEp-2 (Bio-Whittaker, Walkersville, Md.) and the LLC-MK2 and MDCK (Vircell, Granada, Spain) cell lines were propagated independently at a final concentration of 150,000 cells/ml. A mixed cellular suspension containing 50,000 cells of each type per ml was prepared in modified Eagle minimum essential medium (MEM; ICN Biomedicals, Aurora, Ohio) containing 10% fetal bovine serum (FBS). To prepare CoHLM monolayers, 1 ml of this suspension was poured into flat-bottom tubes (16 by 50 mm), each containing one 12-mm-diameter coverslip, and the tubes were incubated at 37°C for 24 h. After incubation, the medium was discarded and was replaced by MEM containing 1% FBS for the maintenance of cellular monolayers.

To assess the effect of age on the ability of CoHLM SV monolayers to detect RVs, 5 RSV, 3 AD, 1 PARA-1, 1 PARA-2, 3 PARA-3, 3 FLU A, and 3 FLU B strains previously isolated in our laboratory were grown in appropriate tissue cultures. The infected monolayers were scraped, and the cells were homogenized in 15 ml of MEM, sonicated for 1 min, and centrifuged at 500 × *g* for 5 min (3). Two aliquots of each strain were separated and were kept in liquid nitrogen until use. One aliquot was inoculated into four vials (200 μl per vial) containing CoHLM that were 24 h old, and the other aliquot was inoculated into four vials containing CoHLM that were 7 days old. The vials were centrifuged at 3,500 × *g* for 15 min (25°C) (3). After adsorption at 37°C for 1 h, the supernatant was discarded, and then 1 ml of MEM containing 1% FBS plus 0.2 μg of trypsin (Sigma Chemical Co., St. Louis, Mo.) per ml was added to each vial and the vials were incubated at 37°C with continuous shaking for 48 h. The cell monolayers that formed on the coverslip in each vial were fixed with −20°C acetone and stained with fluorescein-labelled monoclonal antibodies specific for each virus (IMAGEN; Dako Diagnostics Ltd., Cambridgeshire, United Kingdom). The recount of infected fluorescent cells was performed with 10 fields at ×250 magnification with an epifluorescence microscope. All 19 strains grew in both the 24-h- and the 7-day-old vials, and the sensitivity of the CoHLM culture to viral infection remained stable 1 week after preparation. The variability in the number of fluorescent foci between the 24-h- and the 7-day-old vials was less than 6%.

To evaluate the clinical usefulness of the CoHLM SV assay for the rapid detection of RVs, we studied 358 nasal wash specimens submitted to our laboratory from December 1996 to February 1998. Samples were obtained from children whose ages ranged from 15 days to 4 years and who had been diagnosed with acute respiratory infection.

The nasal wash specimens were sonicated for 1 min and were centrifuged at 500 × *g* for 5 min to obtain a cell-free supernatant (3), and the supernatant was inoculated into TCs and SVs. For the SV assay, two aliquots (0.2 ml each) of the supernatant were inoculated into two freshly prepared CoHLM SVs as described above, and for the TC assay, HEp-2, LLC-MK2, and MDCK cell monolayers were each inoculated with 0.2 ml of sample by previously described methods (16).

After 48 h of incubation, the monolayer of one vial was stained by indirect immunofluorescence with a pool of monoclonal antibodies directed against the seven RVs: RSV, AD, FLU A, FLU B, PARA-1, PARA-2, and PARA-3 (Respiratory Viral Screen IFA; Light Diagnostics, Temecula, Calif.). When fluorescent cells were observed, the cell monolayer of the other vial was scraped and the scrapings were dotted onto seven

* Corresponding author. Mailing address: Servicio de Microbiología, Hospital Universitario Virgen de las Nieves, Avda. Fuerzas Armadas, s/n, 18014 Granada, Spain. Phone: 0034 958 241109. Fax: 0034 958 241282. E-mail: delarosa@cica.es.

TABLE 1. Comparison of results obtained by traditional TC and simultaneous culture in a single SV containing HEp-2, LLC-MK2, and MDCK cells for 358 respiratory specimens

Virus (no. of isolates)	No. of viral isolates with the following result[a]:		
	SV^+, TC^-	SV^-, TC^+	SV^+, TC^+
	3	7	125
FLU A (18)	0	0	18
FLU B (5)	0	1	4
PARA-1 (4)	0	1	3
AD (8)	0	1	7
Total (170)	3	10	157

[a] SV^+, TC^-, positive result by the CoHLM SV assay and negative result by traditional TC; SV^-, TC^+, negative result by the CoHLM SV assay and positive result by traditional TC; SV^+, TC^+, positive results by both methods.

wells of a 10-well slide. Staining with monoclonal antibodies against each virus (IMAGEN; Dako Diagnostic Ltd.) was carried out for final identification. The tubes were observed every 48 h for at least 14 days for detection of the cytopathic effect (CPE). Hemadsorption and hemagglutination were performed in tubes containing LLC-MK2 and MDCK cell monolayers without CPEs before they were considered negative and discarded. Positive isolates were finally identified by direct immunofluorescence staining.

The results of TC and the CoHLM SV assay are summarized in Table 1. One hundred seventy strains from 168 nasal wash specimens were identified: 167 by TC and 160 by the CoHLM SV assay. Three RSV strains were detected only by the SV assay, while seven were detected only by TC. These results were confirmed by immunoassay (TestPack RSV; Abbott). Coinfections with AD and RSV and with FLU A and RSV were detected in two patients by both methods. Overall, the CoHLM SV assay allowed the detection and identification of 95.2% of viruses in 48 h, whereas 98.2% of the viruses were identified by TC in a mean time of 6 days.

Rapid diagnosis of viral respiratory infections has become increasingly necessary for clinicians not only for therapeutic reasons but also for improvement of patient care outcomes such as nosocomial infection control and patient management (1, 8). This would avoid the use of unnecessary antimicrobial therapy, leading to decreases in costs and in the durations of hospital stays (8, 17).

Laboratory methods for identification of the main RVs by cell culture are too slow to provide useful clinical information. The SV assay represents a remarkable advance since it shortens the time needed to obtain results compared with the time required for the traditional TC, although for the SV assay separate tests must be run for each suspected virus (specific cell lines and identification reagents are needed).

The use of monoclonal antibody pools for presumptive identification of viral infections greatly simplifies the SV assay (9, 13, 14). Theoretically, this technique could be improved by using monoclonal antiserum pools labelled with different fluorochromes which would allow final viral identification in a single step (2, 11). These reagents are not easily available, and the need for a fluorescence microscope versatile enough to detect all the fluorochromes makes this approach difficult (11).

The use of monkey kidney primary cells (RMKs) for the SV assay for the identification of RV has been reported (2). This cell line has a wide spectrum of sensitivity to viral infection, although some viruses greatly involved in human pathology, such as AD and RSV, have shown low yields with this cell line (5). On the other hand, RMKs are difficult to manage, and the lot-to-lot variability for RMKs is greater than that for continuous lines.

The results obtained by the CoHLM SV assay are similar to those reported in tests for each virus performed independently (9, 13, 14). Thus, we consider this approach to be useful for laboratories capable of maintaining continuous cell lines, due to its high sensitivity and ease of use. The CoHLM SV assay allows a rapid diagnosis of most acute RV infections by inoculation of each specimen into a single vial that contains a cellular environment sensitive to the seven viruses mainly involved in human respiratory pathology.

Recent studies have reported that other cell lines could be as sensitive as MDCK, LLC-MK2, and HEp-2 cells to RVs (6, 15), although further studies would be necessary to evaluate the application of simultaneous culture in a single vial to other cells.

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