Use of High-Speed Rolling To Detect Respiratory Syncytial Virus in Cell Culture

MICHELE A. STURGILL and JOHN H. HUGHES

Department of Medical Microbiology and Immunology, The Ohio State University, Columbus, Ohio 43210, and Viral Diagnostic Laboratory, Children's Hospital, Columbus, Ohio 43205

Received 21 September 1988/Accepted 6 December 1988

We examined the effect of motion on respiratory syncytial virus (RSV) growth in cell culture. Infected cultures were incubated stationary, rolling, or on an orbital shaker. Enzyme immunoassay (EIA) results for cultures infected with high concentrations of a laboratory strain of RSV were similar for all incubation conditions. However, cultures infected with low concentrations of virus and rolled at 96 rpm had a significantly greater mean EIA optical density (1.78 ± 0.22) than cultures rolled at 2 rpm (1.42 ± 0.08) (P < 0.05). The mean EIA optical density of high-speed cultured was also significantly greater than for cultures on an orbital shaker (1.25 ± 0.08) or for stationary cultures (0.21 ± 0.17) (P < 0.01). The amount of virus measured by EIA from cultures infected with clinical specimens was also found to be significantly greater at 96 rpm than for stationary cultures. Cultures infected with cell culture isolates were detected significantly earlier at 96 rpm than when stationary. We suggest that high-speed rolling can be used to enhance the detection of RSV in clinical specimens, especially if the virus is present in low concentrations.

Respiratory syncytial virus (RSV) is the major cause of lower respiratory tract infection in infants and children (5). Since antiviral agents are now available, rapid viral diagnosis can aid in treatment during the course of infection (13). Because virus isolation in cell culture is time-consuming, more rapid methods of virus detection such as immunofluorescence (1, 6, 8, 9, 18) and enzyme immunoassays (EIAs) are being used in many laboratories (4, 7, 10, 11, 14, 20). However, cell culture isolation remains the standard by which all other methods of virus detection are compared (19). The purpose of this study was to determine the effect of high-speed rolling (96 rpm) and orbital motion on the detection of RSV in clinical specimens, seeking to improve the cell culture isolation method.

RSV (Long strain), clinical specimens in the form of frozen RSV-positive HEP-2 or fibroblast cell culture isolates, and fresh specimens were used for motion studies. Clinical isolates were frozen once the culture became positive and were thawed twice before being used. All studies were carried out with human epidermoid carcinoma type 2 (HEP-2) cells which were seeded into roller tubes (16 by 125 mm) at a density of 80,000/1.5 ml. Cultures were grown to approximately 50% confluency in Eagle minimum essential medium containing 10% fetal bovine serum, 1.2% sodium bicarbonate, and gentamicin (50 μg/ml). Before infection, medium was decanted and cells were refed with Eagle minimum essential medium containing 2% fetal bovine serum, 2% sodium bicarbonate, and gentamicin. Fresh clinical specimens were diluted 1:1 in medium before inoculation into cell cultures. After inoculation with virus, cultures were randomly selected for incubation at 37°C under various conditions (stationary, 2 rpm, 96 rpm, or on an orbital shaker). Cultures were read daily for 5 days for the presence of viral cytopathic effect (CPE). Cultures inoculated with frozen clinical isolates were read and rated blindly. When CPE was observed for a given specimen, usually 2 to 5 days postinfection, infected cultures were frozen and thawed three times. After the last thaw, 0.3 ml of the tissue culture lysate was used in a commercial enzyme immunoassay (EIA) (Kallestad Diagnostics, Inc., Austin, Tex.) to determine the amount of RSV present in the cultures. Optical densities for EIA were measured at 450 nm with a Gifford spectrophotometer.

For rolling at 2 rpm, cultures were placed on a Bellco roller unit. A modified Bellco roller unit (constructed by the Biomedical Engineering group at Children's Hospital, Columbus, Ohio) was used for cultures rolled at 96 rpm and has been previously described (12). For orbital motion at 150 rpm, a stainless steel culture tube rack was taped to the platform of a Bellco orbital shaker (model 7744) so cultures could be incubated in their normal horizontal position.

Differences in RSV antigen concentrations between experimental and control cultures were determined by the Student t test. The Wilcoxon signed-ranks test was used to analyze differences in CPE, detection time, and antigen yields.

To determine the effect of rolling on RSV replication, serial 10-fold dilutions of stock virus (Long strain) were made. Virus was then inoculated into cultures and incubated under the various conditions previously described. The results of RSV antigen quantitation by EIA are shown in Fig. 1. When high concentrations of viruses were used, no significant differences in virus amount were detected. However, when low virus inoculum concentrations were used, significantly more viral antigen was present on days 3 and 4 in cultures rolled at 2 or 96 rpm or rotated on an orbital shaker than in stationary cultures. On day 4, cultures rolled at high speed had a significantly greater mean EIA optical density (1.78 ± 0.22) than those rolled at 2 rpm (1.42 ± 0.08), those rotated on the orbital shaker (1.25 ± 0.08), or stationary cultures (0.21 ± 0.17) (P < 0.01). Cultures placed at 2 rpm or on the orbital shaker also had significantly more antigen on days 3 and 4 postinfection than stationary (control) cultures.

Frozen RSV cell culture isolates were used as inocula to determine the effect of rolling on CPE production. No stationary cultures reached maximum CPE, and only a single stationary culture reached a 3 + CPE rating. However, 13 of 47 cultures (27.7%) rolled at 96 rpm reached 3 + CPE, and 7 of 47 (14.9%) reached maximum CPE. Viral CPE was found...
to be significantly enhanced at 96 rpm, compared with CPE in stationary cultures. The mean CPE for stationary cultures was 0.9, while the mean CPE for 96-rpm-rolled cultures was 2.1 (P < 0.05). EIA quantitation was performed on 13 of 22 frozen specimens that were reinoculated into cell cultures. Absorbance values and CPE ratings correlated (P < 0.01). All reinoculated specimens had greater mean optical densities at high speed, with three specimens having significantly higher optical densities than duplicate stationary cultures. Although a significant difference in RSV antigen formation was not found for all specimens, the syncytia in cultures rolled at high speed were generally more progressed and were more easily detected.

Of the frozen RSV isolates used as inocula, 9 of 22 (41%) were detected 1 to 2 days earlier at 96 rpm, while only 1 of 22 (4%) was detected earlier in stationary cultures (P < 0.05). The remaining specimens (12 of 22 [55%]) were detected on the same day for both stationary and rolled cultures. For five of the 22 specimens (23%), CPE was visually detected only at 96 rpm, while no observable CPE was seen in the corresponding stationary cultures.

Fresh nasal wash specimens were obtained and inoculated into HEp-2 cell cultures. Infected cultures were frozen 2 to 5 days postinfection for EIA quantitation. The amount of virus produced from clinical specimens at 96 rpm (as quantitated by EIA) was significantly greater than that found in stationary cultures. Of seven specimens, all were EIA positive and had a greater mean optical density (P < 0.05 by the Wilcoxon signed-ranks test) under rolling conditions, while only four of seven of the stationary cultures (57%) were EIA positive. More enhanced CPE was again observed at 96 rpm, compared with CPE in stationary cultures.

To our knowledge, no studies have been conducted to determine the effect of motion or rolling at different speeds on RSV detection. Although rolling at low speeds (0.2 to 2 rpm) has been used by some diagnostic laboratories to enhance the detection of viruses in cell culture (3, 16, 17), it has not been reported as a detection method of choice for RSV. We have found that high-speed rolling can augment the cytopathology of RSV in HEp-2 cultures, as evidenced by the significantly greater degree of CPE present in cultures rolled at 96 rpm and by the presence of syncytia with distinct, easy-to-detect characteristics making it easier for a technician to detect RSV in culture. Other advantages for rolling infected cultures at 96 rpm are a reduction in detection time and an increase in virus yields.

While rapid diagnostic tests have proven very useful, not all RSV-containing specimens will be detected by either immunofluorescence assay or EIA (4, 21). Therefore, isolation of RSV in cell culture is still important. Immunofluorescence assays require high-quality reagents and experienced technicians for interpretation of stained slides (2, 20). Cultures that have been rolled should provide a better preparation for RSV detection by EIA or immunofluorescence assay. When cell cultures are used for RSV isolation, our data suggest that cultures should be rolled, and if they are rolled, then at 96 rpm. Similar results have been obtained in our laboratory for herpes simplex virus (15) and vaccinia virus (12).

Further studies are needed to determine the optimum rolling and orbital speed for detecting RSV. It is not yet known how motion is involved in the enhancement of RSV detection or which type of motion is best. Since high-speed rolling can increase the yield of RSV, can augment the RSV cytopathology and can decrease detection time, the cell culture isolation method for RSV is thereby improved and is a better comparison standard for rapid diagnostic tests.

LITERATURE CITED


