Presumptive Identification of Enteroviruses with RD, HEp-2, and RMK Cell Lines

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The limited supply of the Lim Benyesh-Melnick antiserum pools for the typing of enteroviruses has made this test inappropriate for routine use in most clinical laboratories. We studied the correlation between the enterovirus groups and the cell lines on which they displayed cytopathic effect in order to make identifications without using the neutralization test. This study indicated that a presumptive identification of the enterovirus group could be made on the basis of characteristic cytopathic effect derived after passage into rhabdomyosarcoma (RD), HEp-2, and primary rhesus monkey kidney (RMK) cells. Echoviruses and group A coxsackieviruses could be isolated in RD and RMK cells but not HEp-2 cells. Group B coxsackieviruses could be isolated in RMK and HEp-2 cells but not RD cells. Poliovirus could be isolated in all three cell lines. We recommend the use of these cell lines to make presumptive enterovirus group identifications for routine viral isolates.

The World Health Organization has asked for conservative use of the Lim Benyesh-Melnick antiserum pools used in the neutralization test to type enteroviruses (3, 7). This limited supply has led most laboratories to perform the test on a restricted basis.

It has been well documented that each of the four groups of enteroviruses has specific growth characteristics in cell culture (1, 4, 5, 7, 8, 10–13). Poliovirus produces cytopathic effect (CPE) in many cell types, including but not exclusive to primary rhesus monkey kidney (RMK), human embryonic kidney, rhabdomyosarcoma (RD), and buffalo green monkey kidney cells (7). Echoviruses produce CPE in RMK, RD, and human fibroblast cells but not in buffalo green monkey kidney or HeLa cells (4, 7, 12, 13). Group A coxsackievirus is commonly isolated after inoculation into suckling mice, but members of this group have been reported to be isolated in RMK and RD cell lines (with the exception of types A1, A19, and A22) (4, 10, 11, 13). Group B coxsackievirus produces CPE in RMK, buffalo green monkey kidney, and HEp-2 cells but not in RD cells (1, 8, 10, 11).

The distinctive teardrop shape of the CPE is caused by the enteroviruses makes their initial identification easy. Isolation of these viruses can be reported within 5 days of receipt in 62 to 87% of the positive cases (4). However, because of the limited supply of Lim Benyesh-Melnick pools, further typing of the enteroviruses can take months as more laboratories batch their typings.

Over the past 2 years, we have noticed a distinct correlation between the enterovirus group isolated and the cell line on which the differential appearance of CPE occurs after passage. On the basis of these data, we propose a cell culture system incorporating RD, HEp-2, and RMK cells to make a presumptive group typing of enteroviruses based on CPE and cell culture systems.

RD and HEp-2 cells were prepared in our hospital laboratory. The RD cells (passes 100 to 123) were seeded once a week and used before they were 12 days past seeding. HEp-2 cells (passes 377 to 396) were seeded twice a week and used before they reached a confluent monolayer. Primary RMK cells were purchased weekly from Viromed Laboratories (Minneapolis, Minn.).

The positive clinical specimens were collected from a variety of sources (11 throat, 5 nasopharyngeal, 25 cerebral spinal fluid, 18 stool and rectal, 1 pleural fluid, 1 lung biopsy, and 1 vesicle fluid specimen). These specimens arrived at the laboratory in viral transport medium which had been prepared in-house by using Eagle minimal essential medium (MEM) (GIBCO Laboratories, Grand Island, N.Y.), gelatin, and antibiotics. Upon arrival, the specimens were inoculated into a general cell culture system designed for nonspecific viral isolation. This system included two HEp-2, two human foreskin fibroblast, one RMK, and one RD cell culture tube. These tubes were observed daily for CPE.

After the initial detection of typical enterovirus CPE, the culture was frozen at −70°C and 1 mL of the culture medium was split evenly and passed to one RMK, one HEp-2, and one RD tube. These tubes were then observed for CPE for 1 week or until CPE developed.

The presumptive identification of echovirus or group A coxsackievirus was made if the RD and RMK tubes were positive and the HEp-2 tube was negative. A group B coxsackievirus identification was reported when the HEp-2 and RMK tubes were positive and the RD tube was negative. The identification of poliovirus (possibly associated with a recent vaccination) was reported when all three cell lines were positive within the first 3 days of culture. In cases in which only one cell line was positive with characteristic CPE, a report of enterovirus was issued.

Samples positive for enterovirus were also sent to the Wisconsin State Laboratory of Hygiene (Madison) for confirmation and typing by neutralization.

In this study, 62 enterovirus isolates were challenged in RMK, RD, and HEp-2 cell cultures. The cell lines in which these isolates produced CPE are shown in Table 1.

All of the polioviruses (one type 1, three type 2, and one type 3) were isolated in all three cell lines. The two group A coxsackieviruses (one A18 and one A20) were isolated in only RD and RMK cell lines. Likewise, the echoviruses were all isolated in only the RD and RMK cell lines. The echovirus serotypes isolated include types 2 (1 isolate), 6 (1 isolate), 7 (3 isolates), 9 (7 isolates), 11 (1 isolate), 14 (1
isolate), 18 (10 isolates), 21 (4 isolates), 25 (1 isolate), 27 (1 isolate), and 30 (1 isolate). The group B coxsackieviruses were isolated in only the HEp-2 and RMK cell lines, with the exception of one culture. The serotypes of the group B coxsackievirus included B2 (four isolates), B4 (six isolates), and B5 (three isolates).

In 11 cases, the Wisconsin State Laboratory of Hygiene did not type the enterovirus isolate. In three of these cases, they stated that the enteroviruslike agent isolated was untypeable by current available methods but that considering the patterns displayed in the neutralization pools, the agents were likely to be related to the Echo 9 family.

In 8 of the 11 cases mentioned above, the Wisconsin State Laboratory of Hygiene reported that the isolates were enteroviruslike agents, as determined by characteristic CPE in cell culture, and that no further typing would be done because of the limited supply of Lim Benyesh-Melnick antisera. We reported presumptive identification of echovirus or group A coxsackievirus in seven of these eight cases because of their isolation in only the RD and RMK cell lines. The other four cases were reported as enterovirus (three cases in which we could isolate virus only on RMK cells) and as viruslike agent (one case in which we isolated RMK and HEp-2 cells with atypical CPE).

A chi-square analysis ($P < 0.0001$) shows a significant correlation between the enterovirus group and the cell line(s) in which the virus was isolated.

In conclusion, we feel that RD, HEp-2, and RMK cells can be used together to give a presumptive group identification of enterovirus isolates. Given the limited supply of antisera for neutralization tests, an identification based on this cell line analysis is adequate for the physician to provide good patient care.

**LITERATURE CITED**


**TABLE 1. CPE produced by 62 enterovirus isolates in three cell lines**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Peliovirus</th>
<th>Group A coxsackievirus</th>
<th>Group B coxsackievirus</th>
<th>Echo-virus</th>
<th>NT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>RMK</td>
<td>5</td>
<td>2</td>
<td>12</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>HEp-2</td>
<td>5</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

* NT, Not typed or untypeable.