NOTES

Increased Efficiency of Group B Coxsackievirus Isolation from Clinical Specimens by Use of BGM Cells

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A continuous African green monkey kidney cell line, designated BGM, was compared with primary cynomolgus monkey kidney cells and human embryonic lung cells for efficiency of enterovirus isolation. A selective enhanced sensitivity of BGM cells both in terms of isolation rate and speed of isolation was found for group B coxsackieviruses but could not be demonstrated for a number of other nonpolio enteroviruses.

Aseptic meningitis, myocarditis, pericarditis, and disseminated disease of the newborn have been associated with group B coxsackievirus infection (3, 11). These viruses occur seasonally and may account for up to 70% of all enterovirus infections detected in seasons of high prevalence (13). Overall, approximately 33% of all enteroviral disease reported appears to be due to the group B coxsackieviruses (1, 13).

Coxsackie B (CB) virus isolation in cell culture constitutes the primary means of laboratory diagnosis. Conflicting reports have appeared regarding sensitivity of BGM cells, a continuous African green monkey kidney cell line (2), and primary Rhesus monkey kidney (RMK) cells for CB virus isolation (5, 14, 15). Preliminary studies in our laboratory suggested that BGM cells might be a more effective system for detection of CB viruses than primary cynomolgus monkey kidney (CMK) cells. Because most diagnostic laboratories, including our own, now rely on CMK cells for detection of CB virus, we compared CMK and BGM cells to determine which was the most efficient cell system for CB virus isolation from clinical specimens. We report here the superiority of BGM cells for isolation of five of the six CB virus types.

Tube cultures of CMK cells, human embryonic lung (HEL) cells (WI-38 and MRC-5) and human embryonic kidney (HEK) cells were obtained weekly from commercial sources (M. A. Bioproducts, Walkersville, Md.; Flow Laboratories, Inc., Rockville, Md., and HEM Research, Inc., Rockville, Md.). BGM cells were obtained from Gerald Berg, U.S. Environmental Protection Agency, Cincinnati, Ohio, and propagated in our laboratory. Cells between genera-

tions 147 and 176 were used. BGM tube cultures were set up weekly from stock flasks. Before specimen inoculation and at regular intervals thereafter (see below), the media for cell maintenance were changed as follows: CMK and BGM cells with Eagle minimum essential medium containing no serum supplement and HEK and HEL cells with Eagle minimal essential medium containing 10% fetal bovine serum.

A total of 446 routine clinical specimens submitted to the Strong Memorial Hospital-Monroe County Health Department Virus Diagnostic Laboratory during September and October 1980 were tested in parallel. Specimens other than cerebrospinal fluid (CSF) were collected or placed in 2.5 ml of viral transport medium consisting of veal infusion broth with 0.1% gelatin and antibiotics. Specimens in viral transport medium were clarified by centrifugation at $2,000 \times g$ for 10 min before cell culture inoculation. CSF was inoculated without centrifugation. The types of cell cultures routinely used were as follows: for CSF and urine specimens, 1 to 2 tubes of CMK, 1 tube of HEL, and 1 tube of BGM cells; for respiratory and fecal specimens, 1 tube of CMK, 1 tube of HEL, 1 tube of HEK, and 1 tube of BGM cells. Each tube was inoculated with 0.2 ml of clarified specimen. If lesser quantities were available, the inoculum was divided equally among the tubes. All cultures were incubated at 36°C on a roller drum and scored for cytopathic effect (CPE) daily (except Sunday) for the first 7 days and every second to third day thereafter for 14 days. Medium was changed on days 1 and 7 post-inoculation if cultures were free of CPE. Isolates producing typical enteroviral CPE were presumptively

FIG. 1. Characteristic CPE produced by Coxsackie B virus (CB-1) in BGM cells. (A) Uninoculated cells; (B) early focus after 18 h of incubation; (C) involvement of entire monolayer after 48 h of incubation. Magnification, $\times 85$.

identified by neutralization with the intersecting virus antiserum pools supplied by the National Institute of Allergy and Infectious Disease (NIAID) (12). All presumptive identifications were confirmed with monospecific antisera also supplied by NIAID.

Over the 2-month study period, 30 specimens (9 respiratory, 7 CSF, 12 fecal, and 2 urine specimens) were positive for group B coxsackievirus (26 CB3, 2 CB2, and 2 CB1). All were positive in BGM cells, whereas only 60% were positive in CMK cells. Fewer positives were detected in HEK (37%) and HEL (7%) cells. Three CSFs were among the 12 isolates missed in CMK cells. The mean number of days to the first positive CPE for the 30 BGM positive specimens was 1.5 ± 0.5 . Comparison of the mean number of days to the first CPE for positive pairs ($n = 18$) was 2.8 ± 0.9 for CMK cells versus 1.3 ± 0.5 for BGM cells. The overall difference between BGM and CMK cells was significant ($P < 0.01$). CB virus was easily recognized in BGM cells (Fig. 1). CPE began focally and spread rapidly to involve the entire monolayer. Even when simultaneously positive in other cells, CPE in BGM cells was always more extensive and thus more easily recognized. Thirty-one additional specimens were positive for nonpolio enteroviruses (20 positive for echovirus, 11 positive for Coxsackie A virus) during the study period. Whereas all specimens were positive in either CMK or HEL cells or both, only 35% were positive in BGM cells. BGM cells

appeared to be more efficient for the isolation of some serotypes than others; e.g., four of five ECHO-11 and three of three ECHO-22 isolates were positive in BGM cells, but only one of six ECHO-30 and none of four Coxsackie A-16 isolates were detected in BGM cells. Echovirus CPE in BGM cells did not develop as rapidly as did CB virus CPE and, in some cases, was quite indistinct.

Because of what appeared to be a clear superiority of BGM cells for CB3 isolation, we examined their usefulness for isolating each of the five commonly occurring group B serotypes. Forty specimens initially positive in CMK cells for CB1 through CB5 and stored at -70°C for periods varying from 1 month to 4 years were reinoculated into one tube each of BGM cells, CMK cells from Flow Laboratories, and CMK cells from M. A. Bioproducts. In most cases, 0.1 ml of original specimen was inoculated into each tube, but occasionally specimens were diluted with viral transport medium (less than twofold) before inoculation. Tubes were read daily for 10 days.

Of the 40 specimens from which reisolation was attempted, 95% were positive in BGM cells, whereas 70% were positive in CMK cells from each supplier (Table 1). The cumulative mean number of days to the first positive CPE for all serotypes was 1.4 to 1.6 days lower in BGM than in CMK cells. Again, the overall difference between BGM and CMK cells was significant ($P < 0.01$).

TABLE 1. Reisolation of CB virus from clinical specimens

Coxsackievirus	No. of specimens tested	No. of positive specimens ^a			Mean no. of days to positive CPE ^b		
		CMK-MA	CMK-F	BGM	CMK-MA	CMK-F	BGM
B-1	5	4	3	5	3.2	3.7	1.2
B-2	10	6	7	9	2.3	2.3	1.2
B-3	10	9	9	9	2.4	2.4	1.0
B-4	10	7	7	10	2.3	3.0	1.2
B-5	5	2	2	5	2.5	3.0	1.2

^a CMK-MA, Primary cynomolgus monkey kidney cells from M. A. Bioproducts; CMK-F, primary cynomolgus monkey kidney cells from Flow Laboratories; BGM, continuous African green monkey kidney cells.

^b The mean number of days to positive CPE $\pm$ 1 standard deviation was 2.5 ± 0.8 for CMK cells from M. A. Bioproducts, 2.7 ± 1.3 for CMK cells from Flow Laboratories, and 1.1 ± 0.3 for BGM cells.

To determine whether increased CB virus sensitivity might be due to the ability of BGM cells to detect greater numbers of infectious virus particles, two specimens representing each serotype were titrated. Serial 10-fold dilutions were made in viral transport medium and duplicate tubes of BGM and CMK cells were each inoculated with 0.1 ml per dilution beginning at 10^{-1} . The cells were incubated at 36°C on a roller drum and examined daily for CPE for 10 days.

Titers of clinical specimens ranged from <1 to 2.7 and 1.7 to 4 $\log_{10}$ 50% tissue culture infective doses in CMK and BGM cells, respectively (Table 2). In 6 of 10 instances, BGM titers were $\geq 2 \log_{10}$ higher than those in CMK cells, and in all cases higher titers were found in BGM cells.

Over the years, a number of cell systems have been comparatively examined for their abilities to isolate enteroviruses (6, 8–10). Criteria used for comparison include the number of serotypes

isolated, overall isolation rate, highest achievable titers, and, infrequently, speed of isolation. RMK cells emerged as being among the most sensitive cell systems for CB virus isolation and, therefore, have served as the system against which most others are compared. Recently, however, RMK and CMK cells were found to be equally sensitive for enterovirus isolation (4, 7). We chose CMK cells for our comparative studies because the use of CMK cells has supplanted that of RMK cells in most clinical laboratories, including our own.

In our prospective clinical trial, adding BGM cells to the routine cell setup for enterovirus isolation significantly increased our CB virus isolation efficiency both in terms of overall isolation rate and speed. CB viruses were isolated 40% more frequently in BGM cells, and in those instances when both CMK and BGM cells were positive, earlier (1 to 4 days) CPE was noted in BGM cells in 14 of 18 (78%) instances. All CB-positive specimens were detected in BGM cells, and all were positive by day 2 post-inoculation.

We also examined frozen clinical specimens to compare the sensitivity of BGM and CMK cells for isolating each of the five commonly occurring CB serotypes, since only three CB serotypes were isolated and CB3 predominated during the clinical trial. Higher isolation rates, higher achievable titers, and a lower mean number of days to the first positive CPE were observed for CB1 through CB5 in BGM cells. These data suggest that BGM cells may be superior to CMK cells for isolating each of these five CB viruses in the routine clinical setting.

The higher recovery rate found in BGM cells in the reisolation attempts was not completely expected, since previous positive results in CMK cells were used as the basis for selecting specimens. The inoculum size (0.1 ml) used in this phase of our study, however, was at least one-half that employed for initial detection. We believe that the lesser amount of specimen used may have reduced the quantity of virus below that necessary for CMK detection (Table 2).

TABLE 2. Comparative titers of CB virus in clinical specimens

Coxsackie-virus	Specimen type ^a	Titer ^b	
		BGM	CMK
B-1	TS	3.0	1.0
	NP	1.7	<1.0
B-2	NP-TS	2.0	<1.0
	Stool	2.7	<1.0
B-3	NP	2.7	1.0
	TS	3.0	1.7
B-4	Stool	4.0	2.7
	RS	2.7	2.0
B-5	TS	4.0	<1.0
	CSF	3.7	1.7

^a TS, Throat swab; NP, nasopharyngeal swab; RS, rectal swab.

^b $\log_{10}$ 50% tissue culture infective doses per 0.1 ml.

Other investigators examining the relative sensitivity of BGM and RMK cells have demonstrated higher titers of CB virus and increased CB virus recovery rates from wastewater samples in BGM cells (5, 14, 15). Comparable isolation rates, however, were found in RMK and BGM cells in the only survey of routine clinical specimens performed, but only five CB viruses were isolated during the study period (14). Perhaps if more CB viruses had been isolated or if the study had included specimens other than stools which usually contain high titers of virus, the data would more closely resemble that reported here. The limited observations made in our study confirm the relative insensitivity of BGM cells for the isolation of other nonpolio enteroviruses reported by Schmidt et al. (14, 15).

One of our laboratory goals was to obtain virus isolation results in time to affect patient care. In several instances, it was brought to our attention that early reports of virus isolated from CSF had served as a basis for earlier patient discharge and discontinuing antibiotic therapy. In addition, rapid virus isolation and serotype identification by neutralization allowed us to quickly detect the existence and extent of a CB3 outbreak in a neonatal nursery. These benefits were realized through the use of BGM cells during the short clinical trial period (2 months), and they encourage us to believe that BGM cells can be of substantial value to the clinical laboratory, particularly during CB virus outbreaks.

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LITERATURE CITED

1. Assaad, F., and W. C. Cockburn. 1972. Four-year study of WHO virus reports on enteroviruses other than poliovirus. *Bull. WHO* 46:329-336.
2. Barron, A. L., C. Olshevsky, and M. M. Cohen. 1970. Characteristics of the BGM line of cells from African green monkey kidney. *Arch. Gesamte Virusforsch.* 32:389-392.
3. Cherry, J. D. 1976. Enteroviruses, p. 366-413. In J. S. Remington and J. O. Klein (ed.), *Infectious diseases of the fetus and new born infant*. W. B. Saunders Co., Philadelphia.
4. Clark, J., C. Schley, K. Ervine, and K. McIntosh. 1979. Comparison of cynomolgus and rhesus monkey kidney cells for recovery of viruses from clinical specimens. *J. Clin. Microbiol.* 9:554-556.
5. Dahling, D. R., G. Berg, and D. Berman. 1974. BGM, a continuous cell line more sensitive than primary rhesus and African green kidney cells for recovery of viruses from water. *Health Lab. Sci.* 11:275-282.
6. Herrmann, E. C., Jr., D. A. Person, and T. F. Smith. 1972. Experience in the laboratory diagnosis of enterovirus infections in routine medical practice. *Mayo Clin. Proc.* 47:577-586.
7. Hollick, G. E., L. Reichrath, and T. F. Smith. 1977. Comparison of primary rhesus and cynomolgus monkey kidney cell cultures for viral isolation from clinical specimens. *Am. J. Clin. Pathol.* 68:276-278.
8. Hsuing, G. D., and J. L. Melnick. 1957. Comparative susceptibility of kidney cells from different monkey species to enteric viruses (poliomyelitis, coxsackie, and echo groups). *J. Immunol.* 78:137-146.
9. Lee, L. H., C. A. Phillips, M. A. South, J. L. Melnick, and M. D. Yow. 1965. Enteric virus isolation in different cell cultures. *Bull. WHO* 32:657-663.
10. McSwiggan, D. A., and R. George. 1970. Comparison of two cell culture systems for the primary isolation of enteric viruses. *Bull. WHO* 43:295-300.
11. Melnick, J. L. 1976. Enteroviruses, p. 163-207. In A. S. Evans (ed.), *Viral infections of humans*. Plenum Publishing Corp., New York.
12. Melnick, J. L., V. Rennick, B. Hampil, N. J. Schmidt, and H. H. Ho. 1973. Lyophilized combination pools of enterovirus equine antisera: preparation and test procedures for the identification of field strains of 42 enteroviruses. *Bull. WHO* 48:263-268.
13. Nelson, D., H. Hiemstra, T. Minor, and D. D'Alessio. 1979. Non-polio enterovirus activity in Wisconsin based on a 20-year experience in a diagnostic virology laboratory. *Am. J. Epidemiol.* 109:352-361.
14. Schmidt, N. J., H. H. Ho, and E. H. Lennette. 1976. Comparative sensitivity of the BGM cell line for isolation of enteric viruses. *Health Lab. Sci.* 13:115-117.
15. Schmidt, N. J., H. H. Ho, J. L. Riggs, and E. H. Lennette. 1978. Comparative sensitivity of various cell culture systems for isolation of viruses from wastewater and fecal samples. *Appl. Environ. Microbiol.* 36:480-486.