Comparison of Cynomolgus and Rhesus Monkey Kidney Cells for Recovery of Viruses from Clinical Specimens

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Received for publication 19 January 1979

In a study of virus recovery from clinical specimens, cynomolgus monkey kidney cells demonstrated sensitivity equivalent or slightly superior to rhesus kidney cells for isolation of myxo- and paramyxoviruses, adenoviruses, and enteroviruses.

Restricted importation of rhesus monkeys from India has drastically reduced the supply of primary rhesus monkey kidney (RhMK) tissue culture and forced most viral diagnostic laboratories to search for alternative culture types for the recovery of myxoviruses, paramyxoviruses, adenoviruses, and enteroviruses. Primary cynomolgus monkey kidney (CMK) monolayers offer a suitable substrate with sensitivity similar to primary RhMK. The information available on the routine use of CMK cultures in diagnostic laboratories is, however, limited to a single report (1).

Until mid-1976 we routinely inoculated specimens into two primary RhMK culture tubes. During 1976, aware of the impending shortage, we began sporadic use of one RhMK and one CMK tube, and by January 1977 this practice became routine. This report describes our experience with these two tissue culture types.

Roller-tube cultures of primary RhMK were purchased from Flow Laboratories (Rockville, Md.) or Microbiological Associates (Bethesda, Md.). All CMK tubes were purchased from Flow Laboratories. Both types were prepared with simian virus 40 and simian virus 5 antisera and were fed upon arrival in our laboratory with CMRL medium 1969 (Connaught Laboratories Limited, Willowdale, Ontario, Canada) without serum.

All clinical specimens received by the Viral Diagnostic Laboratory at the University of Colorado Medical Center during 1974, 1975, 1977, and the first three months of 1978 were included in this analysis, provided they were inoculated into two tubes of primary monkey kidney (MK) tissue. Most specimens were swabs diluted in veal infusion broth with 0.5% bovine albumin and antibiotics. In addition to MK, specimens were inoculated into diploid human fetal lung fibroblasts and HEp-2 cells.

In all years of this analysis, one of the two

primary MK tissue culture tubes received about twice as much inoculum as the other (usually 8 drops in one tube and 4 drops in the other). This policy was designed to obviate the need for reprocessing of specimens producing toxic effects with large inocula. When two RhMK tubes were used, the first tube received twice as much as the second and the tubes were labeled as such in the laboratory records. When one RhMK and one CMK were used, the RhMK was the "first" tube and received twice as much inoculum as the CMK (or "second") tube.

After inoculation, all tubes were incubated, stationary, at 35 to 36°C and examined for cytopathic effect two or three times weekly. The monolayer in the first tube was hemadsorbed about 1 week after inoculation, according to standard procedures (3). If the result was negative, the medium was changed and the tube was reincubated. The monolayer in the second tube was hemadsorbed after about 2 weeks, the medium having not been changed until then. In certain instances, if the first tube was strongly hemadsorption positive, the second tube was discarded or harvested before hemadsorption. In such instances the specimen was not considered in this analysis. Eighteen percent of all hemadsorption-positive cultures were rejected from the analysis for this reason, 26% during the period 1974-75 and 11% during the period 1977-78.

Viruses recovered in both tubes of a pair were routinely identified from only one tube. Typing and species identification methods were neutralization (parainfluenza virus, enteroviruses, adenoviruses), complement fixation (respiratory syncytial virus, adenoviruses), fluorescence (parainfluenza virus), and hemagglutination inhibition (influenza virus). Under these conditions, recognizably different viruses were never recovered from the two tubes of a pair inoculated with the same specimen.

During 1974-75, when two RhMK tubes were

being used, 4,553 specimens were inoculated and 514 (11.4%) yielded viruses in this tissue. In 1977-78, when one tube of each tissue type was used, 2,968 specimens were inoculated and 466 (15.7%) yielded viruses. This is a significant increase ($\chi^2 = 29.9$, $P < 0.0001$), but it may well have been due to factors other than the change in cell types.

Analysis of the virus recovery rate in the first (RhMK) and second (RhMK in 1974-75, CMK in 1977-78) tubes for all MK-positive specimens likewise suggested the equivalence of CMK cells or, possibly, their superiority. In the earlier interval, 83% of isolates were recovered in the second (RhMK) tube, whereas in the later interval this proportion was 85%. Moreover, when CMK was the second tube used, the recovery rate in that tube alone was about twice the rate in the second RhMK tube in 1974-75 (11% versus 5%, $\chi^2 = 11.7$, $P < 0.001$).

In Table 1 are shown the recovery rates of 10 major viruses or virus groups in each tissue culture tube during the two time periods of the study. There are no significant differences for any of the viruses between the percentage of strains recovered in the CMK tube during 1977-78 and that in the second RhMK tube during 1974-75. On the other hand, significantly more influenza A virus strains were recovered only in CMK than only in the second RhMK tube ($\chi^2 = 9.0$, $P < 0.0005$). Likewise, adenoviruses grew more often only in CMK than only in the second RhMK tube ($\chi^2 = 3.85$, $P < 0.05$).

In several instances the first tube appeared to be significantly more sensitive than the second. Such differences in some instances were probably due to differences in the handling of the tubes (for example, the extra feeding at 1 week

or the larger inoculum in the first tube). They were observed in both time intervals (for parainfluenza 1 and respiratory syncytial viruses) or were of marginal significance (in 1977-78 for influenza B and parainfluenza 3 viruses). An exception was influenza A in 1977-78, where the second tube (CMK) was significantly more sensitive than the first (RhMK) despite the smaller inoculum ($\chi^2 = 4.7$, $P < 0.05$). This difference tends to corroborate the significant differences seen in the second tube comparison above.

Some bias in the analysis might have resulted from rejection of certain cultures from analysis because the second tube was discarded when the first tube was hemadsorption positive, as described above. More cultures were rejected for this reason in the earlier interval (26%) than in the later (11%). Because a rejected tube has a greater-than-random probability of being a first-tube recovery, the remaining nonrejected tubes develop a greater-than-random probability of being second-tube recoveries. Therefore, the bias in the later (low-rejection) interval, in comparison to the earlier (high-rejection) interval, was towards lower second-tube and higher first-tube recovery rates in the later interval. Our findings were, however, if anything the opposite: CMK was more sensitive for influenza A than RhMK, and the overall recovery rate in only the second tube was higher in the later interval.

Two previous studies report comparisons similar to our own, with similar results (1, 2). Only that of Hollick et al. (1) examined a significant number of clinical specimens, and in that study two-thirds of the specimens had been frozen and thawed once and the remainder were taken only from the respiratory tract. Moreover, no parainfluenza 3 isolations were made.

TABLE 1. Comparison of recovery rates in first and second tubes of MK tissue cultures, analyzed by individual viruses isolated

Virus	1974-75 ^a			1977-78 ^b		
	Total no. of viruses isolated in MK	% of strains recovered		Total no. of viruses isolated in MK	% of strains recovered	
		First tube	Second tube		First tube	Second tube
Influenza A	59	97 (10) ^c	90 (3)	55	83 (6)	94 (17)
Influenza B	15	93 (7)	93 (7)	54	98 (11)	89 (2)
Parainfluenza 1	16	100 (38)	63 (0)	40	98 (25)	75 (2)
Parainfluenza 2	21	95 (5)	95 (5)	29	93 (3)	97 (7)
Parainfluenza 3	25	88 (4)	96 (12)	19	100 (16)	84 (0)
Respiratory syncytial	76	92 (46)	54 (8)	80	90 (38)	62 (10)
Echo or coxsackie A	107	93 (7)	93 (7)	43	95 (14)	86 (5)
Coxsackie B	38	97 (5)	95 (3)	32	94 (13)	87 (6)
Poliovirus	86	99 (8)	92 (1)	47	89 (4)	96 (11)
Adenovirus	54	93 (13)	87 (7)	21	76 (14)	86 (24)

^a Two RhMK tubes.

^b RhMK in the first tube, CMK in the second.

^c Numbers in parentheses indicate percentage of viruses recovered only in that tube.

Our own study had certain drawbacks which were to a large extent the result of design dictated by the economic necessities of a laboratory financed entirely through patient fees. Nevertheless, we were able to show that CMK is similar to RhMK for recovery of enteroviruses, influenza A and B, and in small numbers, parainfluenza viruses types 1, 2, and 3. Our study corroborates that of Hollick et al. for myxoviruses and both previous studies for enteroviruses. Furthermore, we have analyzed considerably more data for parainfluenza than were presented by Hollick et al., and we conclude that

CMK cells will probably perform satisfactorily for this group of viruses as well.

LITERATURE CITED

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