Arch. Inst. Razi, 1979,31, 5-8

IMPROVEMENT IN THE YIELD OF ORAL POLIOVIRUS VACCINE (SABIN STRAINS) PRODUCED IN HUMAN DIPLOID CELLS (*)

H. Mirchamsy, A. Shafyi, S. Bahrami, M. Kamali,
P. Nazari and M. Mahinpour

ABSTRACT

When stationary cultures of human diploid cells in conventional Roux bottles were replaced by the development of cells in roller bottles, the yield of poliovirus, Sabin strains types 1 and 2, showed an increase of 0.5 to 1 log 10.

No significant difference was noticed in the yield of these viruses cultured in WI-38 and MRC-5 human diploid cells.

In earlier papers (2, 3), we have described the production of live oral polio vaccine (Sabin strains) and live attenuated measles vaccine in human diploid cells.

As we used WI-38 cells for the production of poliovirus, it was necessary to have sufficient stock of cells for continuous use. We have only received WI-38 cells from the National Institute of Biological Sciences and Control (NIBSC) Hampstead (London) once at population doubling level (PDL) 10. This stock could not last for a long time. Since we knew that the original stock of WI-38 was nearly exhausted, we decided to use MRC-5, the second most well-known human diploid cells (HDC), received twice at PDL 9 from the NIBSC for the manufacture of both measles and polio vaccines. This report is the result of the preliminary study which was carried out in order to compare the yield of poliovirus in WI-38 and MRC-5 cells.

(*) 15th IABS Congress : Vaccination in the Developing Countries. La Guadeloupe 1978. Develop, biol. Standard., vol. 41, pp. 183–185 (S. Karger, Basel 1978).

MATERIALS AND METHODS

Cell cultures

For routine production, batches of 1000 or more WI-38 cells in1 liter Roux-bottles at the 29th to 30th PDL, were made over a period of seven to eight weeks. In order to improve the yield of virus, cells were also grown at PDL 27 or later in roller bottles used by Stones (1977).

Seed virus

Sabin seed viruses at passage level (SO+ 2) for types 1 and 2 and (SO+ 1) for type 3 were used. Details are given in a previous report (3). Virus titration was performed in Vero cells using 10 tubes for each dilution. Virus was diluted at half log basis.

Virus yield in Roux flasks

The summarized results of 20 batches of type 1, 12 batches of type 2 and 10 batches of type 3 are presented in Table 1. Mean virus yield was found to be 7.6, 7.1 and 7.4 TCID50/ml for types 1,2 and 3 respectively.

Table I. Summary of production of live oral polio virus (Sabin strain in WI-38 cells) in Roux flasks

Polio Type	Production Batches	Average Volume (Litres)	Titre(TCID 50/ml) Log 10
1	20		7 6
2	12	30 - 40	7 1
3	10		7 4

Virus yield in roller bottles

Data from our preliminary experiments, presented in Tables II and III showed that the virus yield was consistently higher in roller bottles of both HDC strains compared with stationary cultures. The average titres for type 1 and type 2 was 8.2 and 8.4 for WI-38 and 8.0 to 8.3 for MRC-5 . These results indicate the advantage of the roller system. As a matter of fact, an increase of titre of about 0.5 log 10 for type 1 and 1 log 10 for type 2 was seen.

Table II. Production of polio virus, Sabin strain, type 1 in WI-38 and MRC-5 human diploid cell strains in roller bottles

Experimental Batch No	Seed dilution	incub temp	incub. period (hrs)	Human Diploid Cell Strain WI-38 No of Rollers inoculated	WI-38 No of Rollers Harvested	WI-38 Volume of Harvest (Litres)	MRC-5 No of Rollers inoculated	MRC-5 No of Rollers Harvested	MRC-5 Volume of Harvest (Litres)	Titre(TCID 50) Per ml of Products WI-38	MRC-5
36-1			72	20	20	4 0	20	20	4 0	7 8 5	7 6 2
36-2			6 8	20	19	3 8	18	18	3 6	8 7	8 5 5
36-3			60	19	18	3 6	20	19	3 8	8 1 2	7 8
36-4	1/50000	33±0.5 C°	70	20	18	3 6	20	20	4 0	8 2 5	8 0 5
36-5			72	20	20	4 0	20	19	3 8	8 1 2	8 0
36-6			6 8	20	19	3 8	19	19	3 9	8 2	8 1 5

DISCUSSION

Since conventional stationary cell cutures are not convenient for large-scale manufacture of viral vaccines in human diploid cells, investigators have presented other systems with considerable potential for mass production. Mann and Mucha (1977) have developed the perfused culture system. According to these authors, a single culture vessel of this system has a surface area of 10m2 and would yield 13.5 million doses of type 1 poliovirus. Van Wezel (1977) has

Table III. Production of polio virus, Sabin strain, type 2 in WI-38 and MRC-5 human diploid cell strains in roller bottles

Experimental Batch No	Seed dilution	incub temp	incub period (hrs)	Human Diploid Cell Strain WI-38 No of Rollers inoculated	WI-38 No of Rollers Harvested	WI-38 Volume of Harvest (Litres)	MRC-5 No of Rollers inoculated	MRC-5 No of Rollers Harvested	MRC-5 Volume of Harvest (Litres)	Titre(TCID 50) per ml of products WI-38	MRC-5
36-7			72	40	38	7 6	40	36	7 2	8 9 2	8 4 5
36-8			72	40	38	7 6	20	20	4 0	8 7	8 6
36-9			76	20	18	3 6	40	36	6 9	8 9 2	8 7 5
36-10	1/50000	33±0.5 C°	69	20	19	3 8	38	37	7 3	7 9	7 8 2
36-11			72	19	17	3 3	39	38	7 6	8 0	7 5 4
36-12			72	20	18	3 6	38	38	7 5	8 3	8 4 5

introduced the microcarrier culture technique for large-scale cultivation of human diploid cells. Roller bottles originally proposed for mass production of FMD vaccine in BHK cell line by Nardelli and Panina (1977) have been used by Stones (1977) for the manufacture of oral poliovaccine in HDC. The main problem with perfused or microcarrier cultures is that the number of population doublings of cells cannot be as clearly defined as in monloayer cultures. In the perfusion cultures technique, the virus may undergo a number of replicate cycles in the cells which may lead to the production of a mixed population of virus particles and consequently a higher neurovirulence for monkeys (1). The titre of Sabin polio viruses types 1 and 2 in WI-38 or MRC-5 cells cultured in roller bottles was 0.5 to 1 log 10 higher than in stationary cultures and was not far from the titres obtained in primary monkey kidney cells.

Another conclusion of this study is that there is no significant difference in virus yield between WI-38 and MRC-5.

REFERENCES

1. Mann, G.F. & Mucha, J. de (1977). Replication of polio virus (LSc 2ab) in perfusd cultures of MRC-5 idiploid cells. *Develop. biol. Standard.* **37**, 255-259.
2. Mirchamsy, H., Shafyi, A. et al. (1977). A comparative field trial of five measles vaccines produced in human diploid cell MRC-5. *J. Biol. Standard.* **5**, 1-18.
3. Mirchamsy, H., Shafyi, A. et al. (1977). Experience with production and control of attenuated polioviruses (Sabin-strains)-in human diploid cells. *WHO Working Document BLG/Polio*, pp. 1-10.
4. Nardelli, L. & Panina, G.F. (1977). 10-Years experience with a 28,800 roller bottle plant for FMD vaccine production. *Develop. Biol. Standard.* **37**, 133-138.
5. Stones, P.B. (1977). Production and control of live oral polio virus vaccine in WI-38 diploid cells. *Develop. biol. Standard.* **37**, 251-253.
6. Van Wezel, A.L. (1977). The large-scale cultivation of diploid cell strains in microcarrier culture. Improvement of microcarriers. *Develop. Biol. Standard.* **37**, 143-147.