DISCUSSION

Measles is one of the most ubiquitous human viruses. Its distribution is worldwide. It causes disease in any season and under any climatic conditions. Primarily, it is a disease of children but may become a disease of adolescents and adults in areas where children have been protected with vaccine.

Almost two million children every year in the world, one every 15 seconds, die due to measles. As per sixth EPI (Expanded Programme of Immunization) reports, 10 children die per minute and 10 more are disabled (H. Singh, R. Bhatia). Measles disease alone is responsible for one-eighth of these deaths. Another two million children are permanently disabled by this disease as a result of blindness in association with vitamin A deficiency, deafness or brain damage. These are as a result of encephalitis which occurs as a complication in 1 out of 2,000 cases of measles (H. Singh, R. Bhatia). In developing countries like India, measles is present practically throughout the year. An average age at which children suffer from measles in developing countries is 4-6 years. In India, this age ranges from 1-2 years. About 37% cases in India are in infants (less than 1 year old), about 70% in children below 2 years of age and about 80% in children below 3 years of age. The case fatality rate in India has been reported to be between 1-3%, and among survivors 30% suffer from severe complications (Bhandari et al). Measles and its complications kill almost 2,00,000 children aged less than 5 years every year in India (Garaj R., Chakraborty A.K). This problem of measles is further aggravated because of under-nutrition and poor socioeconomic conditions in developing countries. Malnutrition may interfere with the immune system to measles virus (Gordan, Singh, Wyo).

As a part of a board based push towards the year 1995 and beyond, the EPI has been challenged by the world health
assembly to control measles, the EPI target disease, with the greatest impact on child health. The impact of measles on the lives of millions of young children every year, particularly in developing countries, indicates the urgent need for the continued reduction in the incidence of measles. In light of this, the world health assembly has called for a reduction in measles incidence of 90% from pre-immunization levels by 1995 (WHO/EPI/GEN/92.3). This goal can be reached and sustained through accelerating the Expanded Programme of Immunization (EPI) as a whole.

Before the EPI, there were an estimated 130 million cases of deaths due to measles (WHO/EPI/92.3). Without immunization, virtually all children contract measles. The vaccination programme executed under EPI has resulted in dramatic reduction in both mortality and morbidity due to measles (WHO/92). It is estimated that approximately 80% of the world's children of less than one year of age received measles vaccine in the year 1990. The estimates of measles cases occurring in developing world for the year 1990 were 29 million with 8,80,000 deaths (WHO/EPI/GEN/92). Thus, measles is responsible for more deaths than any other EPI target disease. Complications associated with measles include diarrhoea, pneumonia, otitis media, blindness and encephalitis leaving many children disabled every year. Not only is measles an acute illness, which causes death and complications, but the long-term impact of the infection is also increasingly being recognized. For many months after the acute attack, the effect of an attack by measles can be seen in reduced survival rates of infected infants. The younger the age of infection, the more the noticeable is the effect of this delayed mortality (Singh, Bhatia). The true impact of measles virus infection is much greater than that which is actually recorded in the number of acute deaths generally attributed to measles, making the control of this disease an even greater priority.
As mentioned earlier, in the absence of active chemotherapy and passive immunization, vaccination is the only solution to tackle with the horrifying consequences of this killer disease. The availability of vaccine in almost every part of the world is a prime necessity. To undertake such a huge task of covering millions of children in vaccination programme, the vaccine, its availability, quantity and quality plays a vital role.

The cost of manufacturing this vaccine is a great hurdle in running such programmes. The type of vaccine, its dosage, etc. is already mentioned in the earlier part of this work.

The work programmed and mentioned herein was done under two major objectives. The first objective was to study various methods of growing the cells, the optimization of cell yield, the effect of various inputs on cell yields and the output of virus from various systems. The second objective was to find out the thermostability of currently available vaccine, the verification of various factors affecting the thermostability and finally to develop a methodology for manufacturing a suitable and more thermostable measles vaccine. Both these objectives, when analyzed together, would give a cost effective and technically feasible method of growing the cells, achieving the best possible virus yield and ultimately convert this virus into a thermostable vaccine. This would mean production of a vaccine with high efficiency and cost beneficial manner.

In the initial part of the work (under first objective), the population doubling curve of the cells under study i.e. MRC-5 was established. The aim of this work was to know the population doubling time of the cells. This information was than used to undertake the cell manufacturing activity exactly in a time-bound manner. Having known the population doubling time, a proper predication of growth conditions of the cells could also be done, which is important while infecting these cells with virus. This information was also useful in
arresting the cells in metaphase to study the karyology of these cells.

The next experiment was to know the effect of various types of foetal bovine serum (FBS) on MRC-5 cells. FBS contributes a major or rather decisive cost input in the manufacture of measles vaccine. In many cases, the cell number achieved is directly proportional to the concentration and type of FBS used. An exemplary calculation of the cost for growing the cells is as given below.

Assuming that, starting from one roux bottle (175 cm²) and going through eight population doublings, 128 roux bottles are prepared. Then the important components required and their approximate cost is as follows -

<table>
<thead>
<tr>
<th>Qty. Required</th>
<th>Cost</th>
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<tbody>
<tr>
<td>1) Plastic roux bottles</td>
<td>128 No. Rs. 12,160/-</td>
</tr>
<tr>
<td>2) Minimum Essential Medium</td>
<td>19.2 Lit. Rs. 1,152/-</td>
</tr>
<tr>
<td>3) Foetal bovine serum</td>
<td>1.92 Lit. Rs. 28,300/-</td>
</tr>
</tbody>
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It can be seen from the above calculations that FBS constitutes nearly 68% of the total material cost incurred on the manufacturing of cell. Thus, any saving either by means of use of reduced percentage of FBS or different methods of growing cells means a substantial reduction in the manufacturing cost of measles vaccine.

Keeping these calculations as guidelines in one of the experiments, the optimum concentration of FBS that can be used to get the most optimum results of (MRC-5) cell yield was calculated. It was clear from the experiment that the percentage of FBS can not be brought down drastically. From 10 % FBS we could, at the most, come down to 8 %, but not less than that. This would mean that the cost of FBS in
manufacturing of MRC-5 cells will drop from 68% of the total cost. This reduced percentage of FBS, therefore, will not have a considerable impact on saving of cost of manufacturing of MRC-5 cells. Therefore, followed by this experiment, various technologies for growing MRC-5 cells were tried out.

Growing the anchorage depended MRC-5 cells in stationary culture was the first method of choice. The cells could be grown optimally in plastic roux bottles without any problem with medium consumption data as follows -

The working area of the bottle - 175 cm$^2$

The quantity of medium required - 75 ml.

to cover the surface

Therefore, the requirement of growth medium (i.e. MEM supplemented with 10% FBS) for utilizable growth area comes out to be 0.428 ml per cm$^2$ or in other words, 0.0428 ml of FBS will be required per cm$^2$ of growth area.

There have been several reports of getting better growth in rolling culture than in stationary culture. However, our experience with MRC-5 cells and rolling culture method was not at all encouraging as far as cell yield was concerned.

The next item which was tried out was a completely new development as far as its use for growing MRC-5 cells was concerned. The system (cell factory) is devised in such a manner that it will require less quantity of medium as compared to other stationary cultures and will be easier for scale-up operation. The major drawback of using stationary culture in roux flasks is the tremendous amount of work involved in scale-up operation. To make the manufacturing activity cost effective, we need to produce large quantities of vaccine in smaller infra-structure with less man-power and minimum wastage. Because of the huge requirement of man-power and increased rate of contamination due to increased
number of roux bottles, the use these bottles for scale-up cannot be an easier and beneficial task.

In the development of cell factory, number of disadvantages in roux flask production have been eliminated. The first one is the consumption of medium. A cell factory having an area of 6,000 cm$^2$ requires only 1,600 ml of medium. That means, about 0.266 ml of MEM per cm$^2$ of area. This quantity is about 1.6 times less than that required for growing the cells in roux bottles, or it is 62 % less than the conventional system. This would be a substantial reduction as far as the use of FBS is concerned. The next bigger advantage found out in this system was the same quantity of medium could give us two population doubling of cells which would still reduce the cost of FBS used per cm$^2$ of cell growth area.

The cell factory of 6,000 cm$^2$ area can be easily handled by one person. With respect to surface area, one cell factory is equivalent to 35 roux bottles. Two persons in a day (about 6 hours) can handle about 50 roux bottles converting them to 100 bottles, while in case of cell factory two persons can handled 8 cells factories in a day, which is equivalent to 280 roux bottles.

Therefore, growing the cells in cell factory would save substantially on account of medium, FBS and manpower with less wastage.

The next step in bringing about the improvements in the manufacturing of measles vaccine was the virus yield obtained from various systems. The same types of techniques which were used for growing the cells were also assessed to ascertain their potential for best possible virus yields.

The first experiment for virus yield was done to know the yield of original system (i.e. stationary culture and roux flask). The virus yield was calculated by developing an equation. This equation expresses the yield in terms of million virus particles. In case of multiple harvesting system, different harvest may
have different titre and volumes. Therefore, to know the exact output, it is obvious to take multiplication of titre and volume, expressed in terms of million virus particles. The stationary culture in roux bottles, although a time-tested and original method, has limitations as far as scale-up is concerned.

While calculating the yield of every individual system, another important factor which was considered was the highest titre reached. Measles vaccine in India and abroad is used majority of the times in 10-dose containers. To prepare such 10-dose containers, one has to have a starting bulk titre of minimum 5.2 Log CCID 50 per 0.5 ml. (considering the losses on account of different processes and tests). If a process gives higher titre (e.g. 5.8 to 5.9 log CCID 50 per 0.5 ml) then it is obviously preferred to a method which gives more volume (or more number of harvests) but with less titre. In the later case although the yield in terms of million virus particles is comparable with that of higher titre virus, the quantity of 10-dose bulk generated out of this system becomes a great limitation factor. Therefore, the most appropriate method is the one which gives higher yield as well as more number of harvests.

The stationary culture was followed by the calculation of virus yields in roller cultures. This type of system gave extremely good yield, both in terms of virus yield and highest titre reached. The virus yield per cm$^2$ of growth surface area as well as per cell was also very high. The scale-up problem was to a very less extent as compared to that of stationary culture. Majority of the harvests collected by using this system were consistently having high titre. Since the maximum virus release time was found out, an additional high titre harvest improved the yields of this system to a great extent. Although the rolling culture system did not give us better yields as far as growing of MRC-5 cells was concerned, it gave excellent yields of virus.

The third system which was tried to assess the virus yield was cell factory. The yields of virus in cell factory were not as good
as that obtained in rolling culture method, but were definitely better than in stationary cultures (i.e. roux flasks). The virus yield per cm² of area was also satisfactory. However, the highest titre reached and the number of harvests available for preparing 10-dose bulk were very less. Rolling culture, in such cases, have a tremendous advantage over cell factory.

Based on various experiments carried out for developing the best possible and economical cell culture system and the highest virus yielding system, it was evident that a coupled system consisting of growing MRC-5 cells in cell factory and then transferring it to roller bottles for producing high titre virus would be the best alternative. In this system, MRC-5 cells can be grown at a considerably lower cost (because of less consumption of FBS) with extremely less man-power cost and with a better scale-up facility. The cells thus grown can then be transferred to roller bottles at a final stage. The rolling culture system then would give higher virus yields. Thus, the advantage of growing huge quantities of cells at a considerably lower cost and best possible yield in rolling system can be brought together to get a huge quantity of measles vaccine at appropriate cost.

To ascertain this, the cells grown in cell factory were transferred to rolling culture system and as expected it gave excellent virus yield and thus proved the advantages of a novel method of coupled manufacturing system.

The second part of the work was about the thermal stability of the vaccine. It was observed that the sequence of harvest or the time of harvesting does not affect conclusively the stability of vaccine at least in case of measles vaccine (Edmonston-Zagreb) produced using MRC-5 cells. The use of gelatin with controlled hydrolysis instead of the randomly hydrolyzed gelatin offered excellent thermal stability to the vaccine. This can assure the vaccinee a correct dose of virus and also can reduce, to a considerable extent, the overages that has to be added while manufacturing the vaccine. The reduction in the
amount of virus added would not only be good from the manufacturing side but would also ensure the vaccinee of receiving a minimum required virus antigen, avoiding the overburdening of the immunological system.