Attempts to create active immunity against measles in artificial way go far back in the past. In 18th century, a procedure similar to variolization was developed in Scotland and was named ‘morbillization’ (Home, 1759). In 20th century, attempts at creating artificial immunity have become more intense and have resulted in most different methods of immunization. Thus, children were inoculated with the heat inactivated blood of measles patients or with the phenol treated fluid of cultures of Noguchi type which was believed to contain measles virus (Savini). Other authors tried to inoculate, intranasally, measles virus adapted to chick embryo (O’ Neiel et al). In a similar manner, Rittossa and Mole tried to provoke a modified disease by a virus cultured in chick embryo (Rittossa and Mole, 1941). The Japanese tried to vaccinate children with a virus adapted to chick embryo (Tonigishi et al). Arakawa described the adaptation of measles virus to baby mice after intracerebral inoculation. After a serial passage from mouse to mouse at certain stage of attenuation, it was suggested for vaccine production.

Measles virus was first isolated in tissue culture in 1954 by Enders and Peebles. The name of the strain which was isolated by them was ‘Edmonston’. This development laid down the era of vaccine development against measles. The Edmonston strain, named after the youth from whom the virus was isolated, was used for many of the vaccines developed worldwide. To make the new famous Edmonston B vaccine, Enders and his colleagues further passaged the Edmonston strain at 35°C to 36°C 24 times in primary kidney cells and 28 times in primary human amnion cells. This attenuated Edmonston B vaccine was licensed in the U.S.A. in March 1963. Although the administration of the Edmonston B vaccine was associated with high fever i.e. more than 39.4°C (103°F) in 20 to 40% cases and rash in 50% cases, the recipients remained remarkably
well. However, simultaneous administration of a small dose of immunoglobulin eventually set at 0.02 ml per kilogram (1963) reduced the occurrence of high fever and rash by approximately 50%. Approximately 18.9 million doses of Edmonston B vaccine were administered in the United States between 1963 to 1975. A formalin inactivated, alum precipitated vaccine derived from the Edmonston strain was also licensed in the United States in 1963 and used until 1967. Usually, three doses of killed vaccine (KKK) or two doses of killed and one dose of live vaccine (KKL) were administered at monthly intervals with few side effects (Krugman, 1972). Killed vaccine was eventually not recommended when, as noted previously, it became apparent that this vaccine produced short lived immunity and placed many recipients at risk for atypical measles infection (CDC, M.M.W.R., 1967).

Two further attenuated live measles vaccines derived from Edmonston strain were later licensed in the United States, the Schwarz strain in 1965 and the Moraten strain in 1968.

The development of majority of vaccines strains available today are the derivatives of original Edmonston strain. (Table-I)

The purpose of attenuating a virus is to weaken it by various growing conditions, so that the resultant progeny of virus will lose its virulent characters but will retain its essential antigenic configuration.

For the attenuation of measles virus, different authors used different cell culture such as primary cells of human kidney, human amnion, dog kidney, chick fibroblast and embryonated hen's eggs. The virus multiplication was performed in tissue culture at temperature ranging from 32°C to 37°C. All strains that are being currently used, originate from the first attenuated strain of measles virus, the 'Edmonston' strain.
Enders used primary culture of human kidney (24 passages) and human amnion (28 passages) for the attenuation of measles virus. He passaged his strain before giving it to Hilleman for the production of vaccine first in chick embryo (6 + 6 times) and then in chick embryo fibroblast (13 + 6 times) having thus obtained the strain which he called 'Edmonston B'. (Table-II)

Musser passaged the Edmonston strain which he got from Enders for further 22 times in chick embryo and 15 times in primary dog kidney tissue culture and with this strain he prepared a vaccine (Musser and Slator, 1962).

Some authors considered the post-vaccinal reactions caused by the vaccine prepared using the mentioned strains too strong and to make them milder they attempted at a further attenuation of the Edmonston strain. The further attenuation was done by passing the strain in chick embryo fibroblast (Gofle, 1964; Schwarz, 1964; Milovanovic, 1965). Schwarz passaged the Edmonston strain in chick fibroblast 85 times and it has all the properties of further attenuated virus strain.

It was only Smordiatsev who did not use the Edmonston but his own virus strain, which he had successfully attenuated in primary cultures of guinea pigs at temperature ranging from 30°C to 35°C.

The examinations have confirmed that only the development of further attenuated strain of measles virus solves the problem of post-vaccinal reactions in a satisfactory manner.
Table - I

- DERIVATIVES OF EDMONSTON STRAIN -

EDMONSTON

EDMONSTON VACCINE A

AIK-C

SCHWARZ

EDMONSTON VACCINE B

EDMONSTON ZAGREB

PHILIPS EDMONSTON MUSSER

MORATEN MORE ATTENUATED STRAIN
### Table - II

**ATTENUATION PROCEDURES ADAPTED FOR VARIOUS MEASLES VACCINE STRAINS**

<table>
<thead>
<tr>
<th>Name of the strain</th>
<th>Type of tissue culture</th>
<th>No. of passages undergone</th>
<th>Temp. of growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edmonston A</td>
<td>Human kidney (primary culture)</td>
<td>24</td>
<td>37°C</td>
</tr>
<tr>
<td></td>
<td>Human amnion (primary culture)</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chick embryo</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chick embryo (primary culture)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Edmonston B</td>
<td>Human Kidney (primary culture)</td>
<td>24</td>
<td>37°C</td>
</tr>
<tr>
<td></td>
<td>Human amnion (primary culture)</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chick embryo</td>
<td>6 + 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chick embryo (primary culture)</td>
<td>13 + 6</td>
<td></td>
</tr>
</tbody>
</table>
This led to the development of a generation of strain which was called as ‘Further attenuated measles vaccine strain’. The procedures adapted for further attenuation were passaging the original strain more number of times in a particular host (Chicken embryo culture and human diploid cells) and altering the temperature of growing the virus. This work has resulted in development of 4 further attenuated strains in two groups (derived from Edmonston A and Edmonston B strains) -

**Group I - Strains further attenuated by passaging on chicken embryo fibroblast -**

- a) Edmonston schwarz - 85 passages on chicken embryo fibroblast at 32°C
- b) Edmonston Beckenham 20 - 71 passages in chicken embryo fibroblast at 33°C
- c) Edmonton Mithovansvi - 94 passages in chicken embryo fibroblast at 37°C

**Group II - Strains adapted and further attenuated by passaging on human diploid cells -**

- a) Edmonston Zagreb - 22 passages on human diploid cells (WI-38) at 32°C

The attenuation of a virus through passages in primary cell cultures harbours a potential danger that the seed virus may contain some adventitious virus whose host are the primary cells in which the passages of the virus were being performed. It is a known fact that some poliomyelitis seed viruses contained the simian (SV-40) virus (Swert and Hilleman, 1960) which was later established to belong to the group of oncogenic viruses and to have the ability of transforming the normal human cells (Koprowski et al, 1962). Some other seed viruses attenuated in embryonated eggs and in chicken embryo fibroblast, contained fowl leucosis viruses.
In the effort to bypass the shortcomings of primary cells, human diploid cell strain was selected by many scientists as a new substrate for the production of viral vaccine.

The fundamental principle behind the development of a human diploid cell strain was established by Hayflick and Moorhead in USA. The Wistar Institute - 38 or WI-38, as popularly known, was the first human diploid cell strain from a female foetus. This strain was extensively studied for -

a) Growth characters,

b) Adventitious agents - bacteria, fungi, mycoplasma, viruses,

c) Karyology,

d) Serial cultivation properties,

e) Susceptibility to various viruses, etc.

This was, then, followed by the development of another human diploid cell strain, MRC-5 (Medical Research Council - 5, J.P.Jacob), which is the strain of choice today. This cell strain is derived from a lung tissue of a male foetus (Jacobs, Johns and Bacillie, 1970). This strain is extensively studied thereafter by several workers all over the world for detecting adventitious agents, growth characters, susceptibility to various viruses, karyograms, etc. Since then, this cell strain is found to be free of any adventitious agents and is used for production by many of the vaccine manufacturers (Ikic, Dedic, Juzbasic, Beck). In the process of such production a cell bank of MRC-5 cells at lower passage level (usually at 18th population doubling level) is prepared and kept frozen in liquid nitrogen. The processing and use of MRC-5 cells is done according to the guidelines laid down by WHO (WHO Requirements for biological
substances no. 12, 1987). Usually one ampoule frozen in liquid nitrogen is revived (PDL-18) and then subsequently doubled at each cycle. By this way a huge quantity of cells can be generated at PDL-29 to PDL-32.

The invention of human diploid cell strain and a need to avoid the vaccine associated reaction led to the development of ‘Edmonston-Zagreb’ measles vaccine strain. The purpose of this work was to further attenuate the Edmonston measles virus strain (Enders, 1962) by passaging it in human diploid cell strain (Ikic, Juzbasic, Cak, Beck, and Hecimovic), preserving, at the same time, its satisfactory immunogenic potential of the virus, then purifying and stabilizing it by plaquing it in human diploid cell strain, WI-38. The rational of this approach to the problem of further attenuation of measles virus was as follows.

The passage of the virus in WI-38 cells at 37°C had the purpose to reduce the virulence of Edmonston strain, to further attenuate it and at the same time to make the virus adapted to human diploid cells (WI-38) so that it may give a high titre when used for the production of vaccine in this substrate. Besides the production, passage of the Edmonston virus in a strictly controlled line of human diploid cells (WI-38) as well as the purification of the virus in the same substrate should have freed the measles virus from possible adventitious viruses, so that the seed would represent a homogeneous, genetically stable population of virus particles originating from single plaque (Ikic, Juzbasic, Beck). The purification of virus was done three times during the adaptation procedure at passage level 9, 11 and 13th (Ikic, Juzbasic, Beck, 1973).

In field trials measles vaccine prepared from Edmonston-Zagreb strain clearly differed from that prepared from Edmonston strain regardless of what substrate was used, HDC or CEC (Beck et al). Post-vaccinal reactions caused by the former were mild, showing that the Edmonston-Zagreb measles virus was, indeed, further attenuated.
A global programme of measles eradication is underway through the project named "Expanded Programme of Immunization" (EPI). Intensification of measles control needs to take place within the wider context of an expanded programme of immunization. Under this programme, poliomyelitis has been singled out for eradication by the year 2000 (WHO, 1992). Neonatal tetanus has been targeted for elimination by the year 1995. One of the reasons for targeting measles is that it may be considered as an indicator for the control of other target diseases being the first vaccine to be given in the present EPI schedule. If the incidence of reported cases of measles is markedly reduced, it is most likely that other EPI target diseases are controlled as well (WHO, 1992.3).

It is totally evident from the data mentioned that the vaccination of susceptible population is the only answer to overcome this problem.

It has been observed that the vaccines manufactured using human diploid cell strains are the best possible antigens as vaccines. The basic advantage of this system is, it is a homologous system for the human vaccinees. In case of vaccines available other than HDC (e.g. CEC), they harbour the risk of anaphylactic shock to the persons sensitive to egg protein (if vaccine is produced in chicken embryo fibroblast). Although it is indicated or warned to the vaccinees by the manufacturer of such products, that there exists such a danger of getting anaphylactic shock to the susceptible persons, it is most of the times impossible for a physician to know if the child is susceptible to egg protein or not. Since the vaccination is performed at the age of 10-15 months, the parents may not be aware of such problem. Secondly, these vaccinations in developing countries like India are carried out in camps and at that time there are chances of missing even the reference of such contra-indications to the parents. This problem of anaphylaxis will be more serious in case of two-dose schedule of measles vaccine (two-dose schedule has been adapted by some of the developed countries).
Thus, the vaccine produced on human diploid cells would be a most appropriate antigen. However, the manufacturing of vaccine on human diploid cells have following constraints to get economically viable product. The major cost burden involved in production of this vaccine is under following heads-

1) Plant and machinery

2) Building and infrastructure

3) Man-power

4) High value raw material

PROBLEMS ENCOUNTERED IN LARGE SCALE PRODUCTION OF MEASLES VACCINE -

The manufacture of measles vaccine on human diploid cells on industrial scale involves investment of a big capital. To grow huge quantities of human diploid cells and that too, without any preservative, involves investment of lot of money on proper design of building, air handling system with proper filter arrangements, supply of air-conditioned air through filter, separation of various manufacturing activities like media production, cell growth, virus culture, vaccine blending, filling into containers, lyophilization and sealing etc. Besides the washing and sterilization facility, large number of work force to carry out stringently controlled washing procedure, filtration system, huge ultra-cold storages, distilled water plant, uninterrupted power supply, etc. are the unavoidable elements which add to the cost of production of measles vaccine to a great extent. On the top of it, the raw material required for the production of such vaccine includes items like foetal bovine serum which costs around Rs. 15,000/- per litre (as the prevailing price in 1993), minimum essential medium (MEM), and various amino acids, vitamins, etc. The requirement of
working capital, therefore, is also substantial in venturing such manufacturing activity.

The quality control set up is also a matter of significant expenditure. The requirement for an animal house, a separate building for animal house, mycoplasma testing facility, on-line virus and cell culture testing facility housed in separate earmarked area is a must for completing the production activity.

The filling and lyophilization of the product is also a project in itself. The selection of right kind of containers, stoppers for lyophilization, fast filling machines, big capacity lyophilizers are the essentials of production activity.

All the above description depicts the thing very vividly, that manufacturing of measles vaccine and that too, on human diploid cell strain (which are most fastidious cells requiring the utmost exacting conditions of growth) is an extremely costly, labour and cost intensive proposition. Out of the above mentioned categories of requirements, there is hardly anything which can be eliminated. In India, the price of such products (like vaccine) is controlled by DPCO (Drug Price Control Order), since this medicine assumes a category of life saving drug. This makes the entrepreneurs more difficult to go for such manufacturing ventures, because from one side, a large amount of money is required to set up the plant and for running it continuously and on the other hand, the higher limit of price he can get is controlled by the regulatory authorities. The only room, then, left for such manufacturing unit is to increase the yields of virus against the standard unit expenditure which can dilute the burden of this heavy cost of production. The optimum utilization of raw material to get higher yield can be achieved by employing the recently developed techniques of culturing animal cells to increase the rate of production, improve the yield and reduce the manpower expenditure. This forms the first part of the work mentioned in the present work, i.e. use of various techniques of
production to optimize the level of production or in other words, improve the existing techniques of production to the fullest advantage to bring down the cost of production, keeping the quality control parameters as per the guidelines of ‘World Health Organization’.

There is another facet of this problem. Before going into the logic of tackling this problem, we will have to see some of the requirements for the product to pass the international norms. These requirements are as follows -

1) The minimum immunizing dose of measles vaccine, live, is 1000 virus particles per human dose (WHO biological substances no. 12, 1987).

2) There is another requirement of thermostability of the vaccine, which states that, the product (lyophilized measles vaccine, live; WHO Weekly Epidemiological Record, No. 23) after heating at 37°C continuously for 7 days, will retain the potency of 1000 virus particles per human dose and the difference between the virus content of heated (37°C, 7 days) and unheated (kept at 4°C) of the same lot should not be more than one log. The product is supposed to comply both the conditions simultaneously. Besides this, there is some loss of virus activity in the process of lyophilization to the tune of 0.2 Log CCID 50/0.5 ml. So, these losses have to be accounted for, before initiating the process of lyophilization. Because of the requirement of thermostability, for the product to qualify the minimum conditions of potency, the manufacturer has to add extra virus before the process of lyophilization. The usual loss on account of heating of product at 37°C for 7 days is 0.6 log to 0.8 log. (As per WHO guidelines it should not exceed 1 log).

3) The assay system of virus content is the titration of live virus on the susceptible cell lines. The test inherits some variation among different laboratories. The manufacturer has to add
some extra quantity of virus considering the titration results of the regulatory authorities which might, sometimes, be lower than that expected. In the event of the product assessed by the third agencies, the test for virus content should be well within the limits of passing.

Considering all these points, we can establish an equation as follows -

a) The required immunizing dose $\quad - 3.0 \log$

b) The losses on account of process of lyophilization $\quad - 0.2 \log$

c) The losses on account of thermostability test to pass- $\quad 0.8 \log$

d) Margin for variation in the titration procedures $\quad - 0.2 \log$

<p>| | |</p>
<table>
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<tbody>
<tr>
<td>Total</td>
<td>$- 4.2 \log$</td>
</tr>
</tbody>
</table>

This means, to get ultimately one dose equivalent of virus content in the finished product (which is $1000$ virus particles i.e. $10^3$), the manufacturer has to start with $4.2 \log$ virus particles.

Viewing at the overage that one has to add to get the standard quality product, it can be concluded that, to bring down the overages to be added, the only way left out is to decrease the difference between the titre of unheated and heated vials of the same lot or in other words, if the product is made more heat stable, then, obviously, the difference ($0.8 \log$) between heated and unheated vials could be brought down to, say, $0.4 \log$. This means, the increase of production by three-fold, or reducing the cost per unit to that extent. The second part of the work deals with development of a more thermostable vaccine, which is also essential for ensuring the vaccinnee receiving the correct immunizing dose.
So, the total work of improving methodology and quality of the product is studied under two parts.

First part of the work consists of evaluating various techniques of production for their suitability for maximum production and lower the cost of production maintaining the same quality of the vaccine.

Second part of the work deals with attributing more thermostability to the available product for reducing the overages one has to add to qualify the product of the suitable potency all throughout its period of storage.