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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Development of a measles vaccine production process in MRC-5 cells grown on Cytodex1 microcarriers and in a stirred bioreactor

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Abstract Measles vaccination remains the most efficient way to control the spread of the virus. This work focuses on the production of a measles vaccine using stirred conditions as an advanced option for process scale up. Non-porous Cytodex 1 microcarriers were used to support MRC-5 cell growth in suspension cultures. Virus replication was first optimized in spinner flasks, and the effects of various operational parameters were investigated. Cell infection with AIK-C measles strain at an MOI (multiplicity of infection) of 0.005, without glucose regulation and in M199 medium, resulted in a virus titer of $10^{6.25}$ $TCID_{50}$ (median tissue culture infective dose)/ml. To optimize the production process in a 7-l bioreactor, we carried out various perfused cultures using minimum essential medium (MEM)+5% FCS diluted with phosphate-buffered saline (PBS). We achieved a high cell density level (4.1×10^6 cells/ml) with an efficient use of the medium when MEM+5% FCS diluted with PBS at 25% was used during the cell amplification step. Optimization of measles production in MRC-5 cells grown on Cytodex 1 beads in a 7-l bioreactor showed that perfusion was the most efficient when compared to repeated-batch culture. Perfusion at a rate of 0.25 V (reactor volume)/day showed the highest specific productivity (1.6 IVP [infectious virus particle] $cell^{-1}$ day^{-1}). Testing of several stabilizers containing pharmaceutically improved components such as sugars, amino acids, and charged ions showed that the formulation composed of sucrose and $MgCl_2$, led to the maintenance of the infectivity of the AIK-C measles virus strain to a significant level, when stored at +28 °C, +4 °C and −60 °C.

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Introduction

Measles virus belongs to the genus Morbillivirus of the family Paramyxoviridae, order Mononegavirales and consists of 15,894 nucelotides, coding six structural proteins. The nucleoprotein (N), phosphoprotein (P), large protein (L), and genome RNA constitute the ribonucleopprotein complex (RPN). Two glycoproteins of hemagglutinin (H) and fusion (F) are present as envelope spikes and are responsible for virus attachment to the host cell. The M protein acts in virus assembly and maturation (Griffin and Bellini 2001).

Measles is a childhood rash disease associated with substantial morbidity and mortality and was an early target for vaccine development. Measles vaccines have been available since the early 1960s (Ohtake et al. 2010). Measles may be ultimately responsible for more pediatric deaths than any other single agent because of complications from pneumonia, diarrhoea and malnutrition. Measles is also the major cause of preventable blindness in the world, affecting the same disadvantaged populations.

Prior to the widespread of measles vaccine, measles was estimated to result in 5–8 million deaths each year. The decline in mortality from measles in developed countries can be attributed in part to improved nutrition and medical care, but mostly to effective delivery of measles vaccine. However, in resource-poor countries, measles remains the second most common cause of vaccine-preventable deaths in children. This is due in part to the difficulty with delivery of two doses of vaccine to a high proportion of the population and to the inability to effectively immunize

infants under the age of 9 months, leaving a window of vulnerability to infection (Moss and Griffin 2006).

Measles infection therefore remains a heavy public health burden worldwide especially in developing countries. World Health Organization's (WHO) statistics indicate that global measles deaths reached 345,000 in 2005, primarily because of underutilization of the vaccine. The cause of death is predominantly measles pneumonia, making it one of the most common respiratory diseases leading to death in children (Wolfson et al. 2007).

Vaccination against measles is one of the most cost effective health interventions available and one of the most powerful tools for providing equity to poor children. Live attenuated measles vaccines have been used for decades, they show high seroconversion rates and provide long-term protection by inducing both humoral and cell-mediated immunity (Bautista-Lopez et al. 2000; Griffin 1995).

Measles vaccines in current use are produced by replicating an attenuated virus strain in a cell line. The virus can be cultivated in either chick embryonic fibroblasts (Griffin and Pan 2009; Nechaeva et al. 1998; Sidorenko et al. 1989) or in human diploid cell lines such as MRC-5 cells (Cutts et al. 1997).

Most of licensed measles vaccines are produced in stationary culture systems such as roller bottles and cell factory (Aunins 2000). However, these culture systems have several limitations: a non-uniform cell growth pattern (Aunins et al. 2003), a finite surface area, etc. Moreover, the scale-up can only be achieved by increasing the unit number of bottles, which is extremely labour-intensive and susceptible to contamination (Butler 1987). Modern facilities have overcome these shortcomings by the use of expensive robots; however, it is still not possible to control environmental conditions such as pH or dissolved oxygen levels (Warnock and Al-Rubeai 2006)

Microcarrier technology offers an attractive alternative, it is already considered as the most predominant technique for large-scale production of various biologicals by anchorage-dependent mammalian cells. The use of microcarrier-based production systems has been described for several processes up to a production scale of 1 m^3. Numerous virus vaccines like polio (Montagnon et al. 1984), rabies (Trabelsi et al. 2005, 2006) and foot and mouth disease (Radlett 1987) are produced at industrial scale in microcarrier-grown cells.

The benefits of using microcarrier culture for vaccine production are numerous including cost savings in labour, process consumables and laboratory space, and the possibility of greater yields through a better process control and a higher available surface area per unit reactor volume (Tree et al. 2001).

Nevertheless, despite these advantages, few attempts have been reported in the literature describing MRC-5 cells growth on microcarriers for measles virus production. In the present work we studied measles virus production in MRC-5 cells grown on Cytodex 1 microcarriers in a 7-l bioreactor. Our ultimate goal is to develop an easy scalable process for the production of a live attenuated measles vaccine with a high productivity. We first optimised virus production in spinner flasks; we had particularly investigated the effect of multiplicity of infection (MOI), glucose regulation at 1 g/l and culture medium. Then we optimized the process of measles virus production in MRC-5 cells cultivated on Cytodex 1 microcarriers in a 7-l bioreactor. We first investigated the effect of diluting the recirculation medium (MEM+5% FCS) with different amounts of PBS on cell growth and glucose and glutamine metabolism. Then the impact of the operation mode (repeated batch, perfusion) on specific virus productivity was studied. Finally the stability of virus suspension using various stabilizers containing various pharmaceutically approved components was investigated.

Material and methods

Cell line

MRC-5 cells were obtained from the ECACC (Salisbury, UK), at passage 15.

Virus strain

AIK-C virus strain was kindly provided by Institut Razi (Teheran, Iran). The strain was initially obtained from Kitasato Institute (Japan).

Culture media and reagents

Media and foetal calf serum (FCS) were supplied by Invitrogen (Glasgow, UK). Reagents were purchased from Sigma Aldrich (St Louis, USA).

Microcarrier preparation

Cytodex 1 from GE HealthCare (Uppsala, Sweden) were used throughout this study. They were prepared and sterilized according to the manufacturer instructions.

Spinner cultures

MRC-5 cells were first grown in MEM+5% FCS and 3 g/l Cytodex1. Once cell density reached its maximum, cells were washed twice with the virus production medium and infected by the AIK-C measles virus strain with an MOI ranging from 0.01 to 0.005. Cell growth and virus production phases were performed at 37 °C and 34 °C,

respectively. Cultures were carried out in 250 ml spinner containing 200 ml of culture medium. Agitation rate was equal to 30 rev/min, pH was maintained at 7.2 (cell growth phase) or 7.4 (virus production phase) by daily addition of $NaHCO_3$ at 88 g/l. Samples were taken daily to determine cell concentration, cell infection, virus titer and pH. All assays were performed in duplicate.

Bioreactor cultures

Cultures were performed in a 7-l bioreactor (BioBraun, Germany) containing 3 l as a working volume, equipped with a pitch blade impeller and a spin filter (pore size: 75 μm) fixed on the axis. During the cell proliferation step, the following conditions were maintained: pH=7.2 (regulated by CO_2 sparging or addition of $NaHCO_3$ at 88 g/l), pO_2 at 50% air saturation by a continuous surface aeration and direct air sparging when required, temperature at 37 °C and agitation rate at 125 rev/min.

To inoculate the bioreactor, cells were first grown in monolayer in MEM+5% FCS at 37 °C, 5%CO_2. Once cells are confluent, they are detached with trypsin according to standard protocols then used to start the bioreactor culture.

Recirculation was started 1 day after the start of culture. Recirculation rate was modulated according to cell density and was gradually increased during the culture as described in Trabelsi et al. (2005). The ratio "recirculation medium volume/bioreactor working volume" was equal to 3.

For measles virus production, pH was maintained at 7.4, pO_2 at 30% air saturation, agitation rate at 125 rev/min and temperature at 34 °C. Once the temperature reached 34 °C, recirculation and agitation were stopped and microcarriers were let to settle down. Cells were then washed twice with M199 and infected at the required MOI in 50% of the working volume. Two hours after cell infection, the bioreactor volume was adjusted to 3 l and the perfusion was restarted at the required rate. The virus was continuously harvested.

For infected cultures conducted in repeated-batch mode, cells were first grown in recirculation then infected as described above. After cell infection, harvests were periodically collected at an interval of 3 days. Each harvest corresponds to 80% of the bioreactor working volume.

The feeding medium was kept at room temperature, whereas the virus harvests were collected at refrigerated temperature (+4 °C).

Samples were taken daily to determine the following parameters: cell density, cell viability, virus titer, glucose, lactate, glutamine and ammonia levels. During cell growth phase, residual glucose level was daily adjusted to 1 g/l. Specific medium consumption rate (l/10^6 cells/day) was evaluated by taking into account culture time and the quantity of medium consumed to achieve the maximum of cell density.

Stability test

Virus harvests were clarified through 8-μm filter then subjected to 0.22-μm filtration. Different stabilizers (Table 1) were added to the harvests. The vaccines were stored at different temperatures: at +28 °C, +4 °C and −60 °C. The stability of each vaccine was assessed by the estimation of virus titer using the tissue culture infectious dose technique, as detailed below.

Cell counting

Aliquots of 5 ml of Vero cell culture were washed three times with PBS, then treated in 5 ml of 0.1 M citric acid containing 0.1% crystal violet and 0.1% Triton X-100 and incubated at least for 1 h at 37 °C. The released nuclei were counted using a hemacytometer.

The specific growth rate, μ (h^{-1}) was estimated by the following equation:

$$\mu = (\ln X_n - \ln X_{n-1}) / (t_n - t_{n-1}),$$

where X represents the viable cell density per milliliter, t represents the time points of sampling expressed in hour, and

Table 1 Composition of measles vaccine stabilizers used in this study

	Stabilizer 1 (S1)	Stabilizer 2 (S2)
Buffer	50 mM Tris–HCl	PBS with Ca^{2+} and Mg^{2+}
pH	7.4	7.2
Sugar (% (w/v))	Sucrose (5)[a]	Lactose (4)
		Sorbitol (2)
Amino acids (mM)	–	Histidine (20)
		Arginine (20)
		Methionine (20)
		Alanine (20)
Divalent ions (mM)	Mg Cl_2 (2)	–

[a]Values between brackets indicate the final concentration in the vaccine

–, Absence

the subscripts n and $n-1$ stand for two succeeding sampling points.

Metabolite analysis

Glucose and lactate concentrations were monitored by enzymatic assays, using specific assay kits from Chronolab (Switzerland, Cat. No.: 101-0014, 101-0040 for glucose and lactate, respectively). Ammonia was quantified enzymatically by the UV-test Cat. No.: 171-UV (Sigma, St. Louis, MO, USA).

Measles virus titration

Measles virus was titrated in microplates on Vero cells as described by Ohtake et al. (2010). Briefly serial dilutions of virus suspension were added to a 96-well plate containing fully confluent Vero cells grown at a cell density of 1×10^5 cells/ml. After 7 days of incubation at 37 °C and 5% CO_2, the plate was examined under a microscope to enumerate the number of wells exhibiting cytopathic effect (CPE). The number of CPE positive wells was then converted to $TCID_{50}$ titer using the Karber method. Virus titer was expressed in $TCID_{50}$/ml.

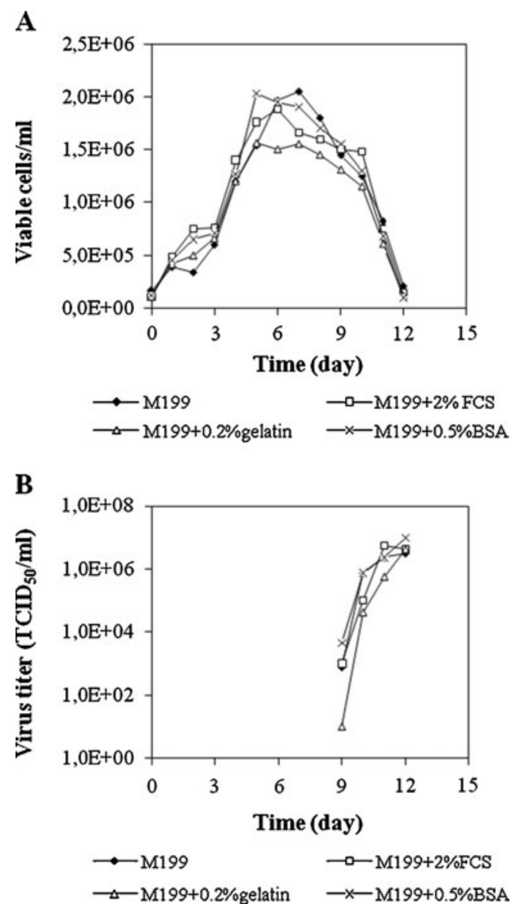

Fig. 1 Kinetics of measles virus production in MRC-5 cells grown in spinner flasks on 3 g/l Cytodex 1. Cells were infected at day 7 at an MOI of 0.01 with AIK-C virus strain, in M199 supplemented with 2% FCS, 0.2% gelatin and 0.5% BSA. Time course of cell density (**a**) and virus titer (**b**)

Results

Optimisation of virus replication in spinner flasks

To optimise measles virus production in MRC-5 cells, the effect of regulating the glucose level at 1 g/l, culture medium and the MOI, were investigated.

Cells were first grown in MEM+5%FCS, on 3 g/l Cytodex 1. Once the cell density reached the highest level (around 1.5 to 2×10^6 cells/ml), cells were washed twice with the medium to be tested: M199, M199+2% FCS, M199+0.2% gelatin and M199+0.5% BSA (Bovine serum albumin). Cells were infected at day 7, at an MOI of 0.01 with AIK-C measles virus strain.

After cell infection, we observed a continuous decrease of cell density, concomitantly virus titer increased. The maximal virus titer was reached 4 to 5 days after cell infection and was equal to $10^{6.5}$, $10^{6.75}$, $10^{6.62}$ and 10^7 $TCID_{50}$/ml, in M199, M199+2% FCS, M199+0.2% gelatin and M199+0.5% BSA, respectively (Fig. 1).

Based on these data, it appears that the enrichment of M199 medium with various components did not result in a dramatic increase of virus titer, and comparable yields were observed for all conditions. Therefore, M199 medium was considered as an acceptable medium for measles virus replication in MRC-5 cells and was chosen for further optimization studies.

The effect of regulation of glucose level at 1 g/l during cell infection phase is illustrated in Table 2. We achieved a maximal virus titer of $10^{6.75}$ and $10^{6.875}$ $TCDI_{50}$/ml, for the regulated and non-regulated glucose level conditions, respectively. The specific productivity was equal to 0.64 and 1.14 infectious virus $cell^{-1}$ day^{-1}. Hence, it appears that maintaining glucose concentration at 1 g/l during cell infection phase did not enhance virus replication.

To study the effect of MOI on measles virus productivity in MRC-5 cells, three levels (0.01, 0.001 and 0.005) were tested. Cells were first grown in MEM+5% FCS on Cytodex 1 at 3 g/l, when the maximal cell density was achieved (around day 5); cells were washed twice with M199 and infected at the required MOI.

Data shown in Fig. 2 show a typical behaviour of cells upon their infection, i.e., a continuous decrease of cell density with a gradual increase of virus titer. The highest virus titer was obtained at an MOI=0.01, 7 days post infection and was equal to $10^{6.875}$ $TCID_{50}$/ml. The use of an MOI of 0.005 resulted in a maximal virus titer of $10^{6.25}$

Table 2 Measles virus production in MRC-5 cells grown on 3 g/l Cytodex 1 in spinner flasks

	Cell density level at infection (cells/ml)	Time to reach maximal virus titer	Maximal virus titer ($TCID_{50}$/ml)	Specific productivity (IVP $cell^{-1}$ day^{-1})§
Regulation of glucose level at 1 g/l	2.2×10^{6}	4 dpi	$10^{6.75}$	0.64
Non-regulation of glucose level	2.2×10^{6}	3 dpi	$10^{6.875}$	1.14

Cells were infected at day 5 at an MOI of 0.01, in M199. Before infection, cells were washed twice with M199 medium

dpi days post infection, *IVP* infectious virus particle

$TCID_{50}$/ml. For the lowest MOI (0.001), the maximal virus titer reached $10^{5.875}$$TCID_{50}$/ml. Therefore, it appears that cell infection at an MOI of 0.005 can be used without a significant loss of cell productivity. Hence, this MOI was selected for bioreactor culture optimization.

Process optimization in a 7-l bioreactor

To develop the process of measles virus production in MRC-5 cells grown on Cytodex 1 microcarriers in a stirred bioreactor, we first optimized cell growth phase then the virus replication phase.

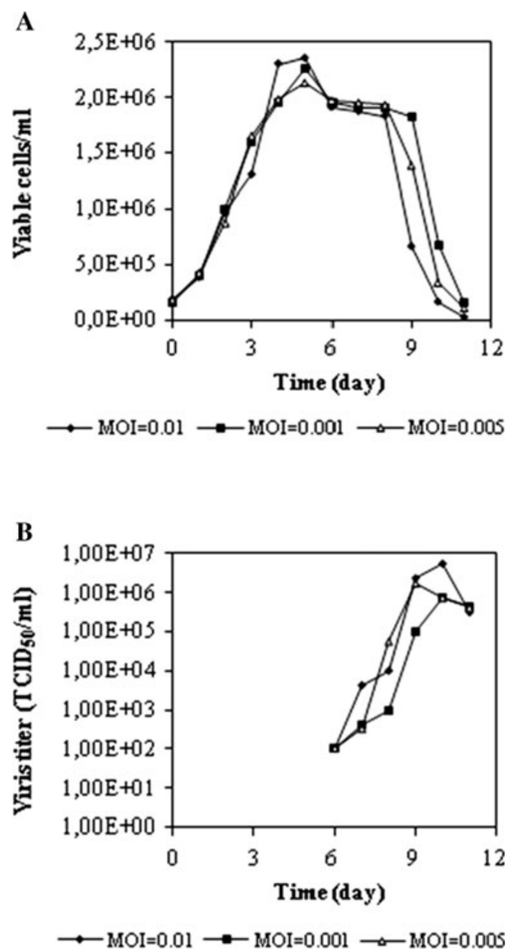

Fig. 2 Effect of the MOI on measles virus production in MRC-5 cells. Cells were grown in spinner flasks on 3 g/l Cytodex 1, and infected at day 5 with different MOI, in M199 medium. Time course of cell density (**a**) and virus titer (**b**)

Cell growth phase

To optimize MRC-5 cells growth on Cytodex 1 microcarriers in a 7-l bioreactor, we first studied the effect of culture medium. MEM was used as a basal medium; in addition, MEM+5% FCS diluted with PBS to 75% and 50% were assessed for their ability to sustain MRC-5 cell growth. The initial glucose and glutamine levels were equal to 3 g/l and 4 mM, respectively.

Data depicted in Table 3 show that the highest cell density level was reached when non-diluted MEM+5% FCS was used as a growth medium. Under these conditions, the cell density level was equal to 5.2×10^{6} cells/ml. We achieved 4.1×10^{6} and 3.4×10^{6} cells/ml, when MEM+5% FCS medium was diluted with 25% and 50% PBS, respectively. However, the highest average specific growth was reached in the 25% PBS-diluted medium. The average specific growth rate (μ_{avg}) observed in the non-diluted medium was slightly lower (0.03 versus 0.034 h^{-1}). In addition, data shown in Table 3 indicate a similar level of the specific medium consumption rate for the 25% PBS-diluted and the non-diluted media. For the 50% PBS-diluted medium, the specific medium consumption rate was slightly lower in comparison to the other conditions. Nevertheless, in this case cell density level was 0.8-fold lower than that observed in 25% PBS-diluted medium. Metabolites levels were comparable for all the media, although the lowest lactate level was obtained in the 25% PBS-diluted medium. Regarding glutamine profile, a limitation was observed on day 5 of the culture, for the 50% PBS-diluted medium (data not shown).

The analysis of these data shows that 25% PBS-diluted medium enables to achieve a high cell density level with an efficient use of the medium. This medium was therefore selected for subsequent optimization studies.

Virus replication phase

To optimize the virus production phase in MRC-5 cells grown on 3 g/l Cytodex 1 microcarriers, the effect of the culture mode and perfusion rate were investigated.

Table 3 MRC-5 cells growth in a 7-l bioreactor under different growth conditions

	Culture 1	Culture 2	Culture 3
PBS dilution (%)	0	25	50
FCS level (%)	5	3.75	2.5
Maximal cell density (10^6/ml)	5.2	4.1	3.4
Average specific growth rate, μ_{avg} (h^{-1})	0.030	0.034	0.025
Lactate level (mM)	14.9	10.6	15.7
Residual glutamine level (mM)	1.84	0.70	0.14
Ammonia level (mM)	2.8	2.7	2.8
Specific medium consumption rate (l/10^6 cells/day)	0.11	0.11	0.08

Cells were grown on 3 g/l Cytodex 1, in recirculation culture mode

Various cultures were conducted using either perfusion or repeated batch as a culture mode during the virus production phase. Cell growth step was always conducted in recirculation in 25% PBS-diluted MEM+5% FCS.

Figure 3a indicates that the highest cell density was obtained at day 7, and was equal to 4.3×10^6 cells/ml. At this moment, cells were infected at an MOI of 0.005; the culture was then conducted using either perfusion or repeated-batch culture mode. After cell infection, a continuous drop of cell density was observed, while we observed a progressive increase of the virus titer, for both cultures. The highest titer was equal to 10^7 $TCID_{50}$/ml and 7.5×10^6 $TCID_{50}$/ml, for the perfusion and the repeated-batch

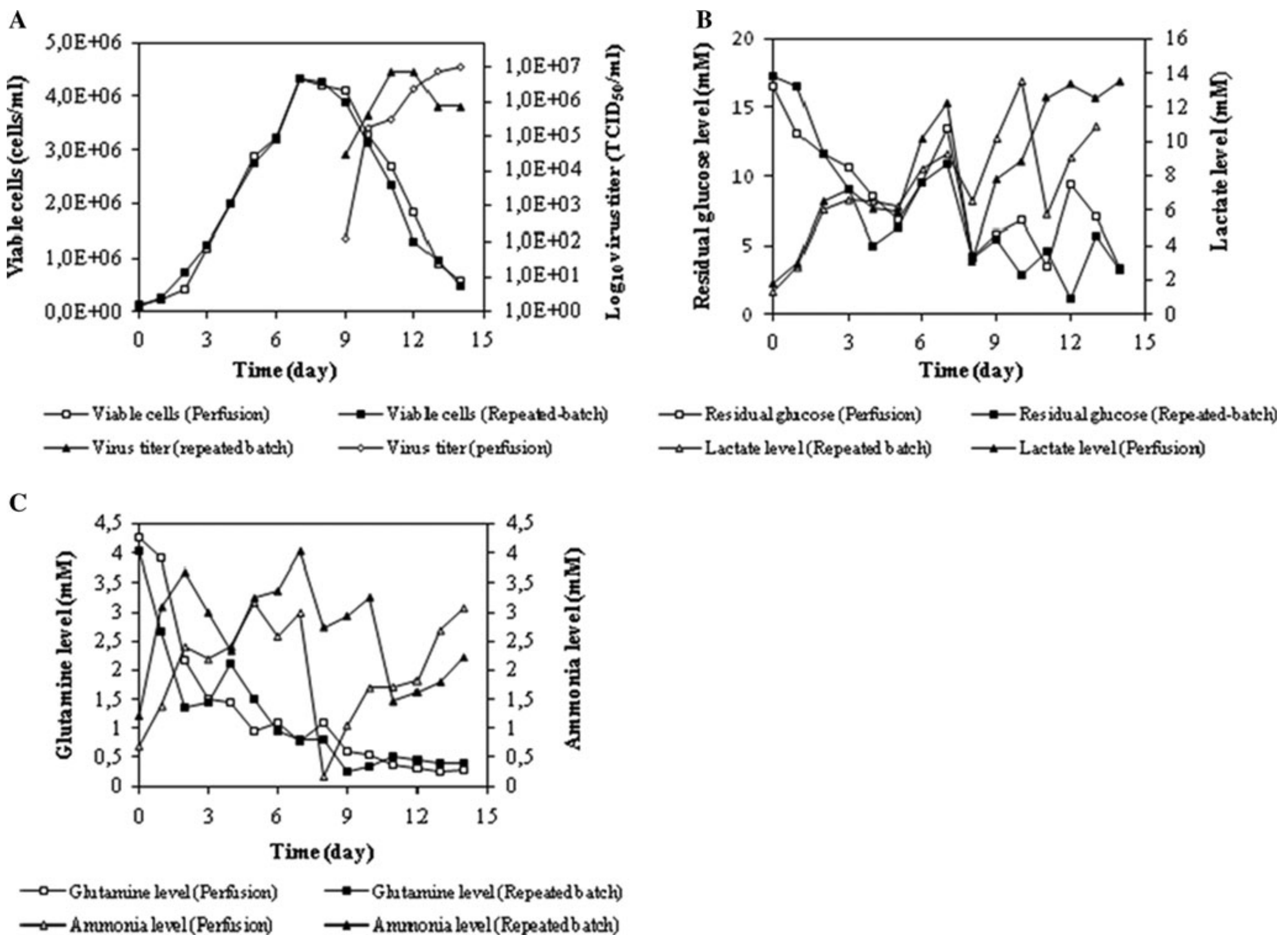

Fig. 3 MRC-5 cells growth and measles virus production on 3 g/l Cytodex 1, in a 7-l bioreactor. During cell growth phase, cells were first grown in batch mode in MEM+5% FCS then in recirculation mode using the same medium but diluted with 25% PBS. Virus production was carried out in M199 using either perfusion or repeated batch culture mode. Cells were infected at day 7 at an MOI of 0.005. Time course of cell density and measles virus titer (**a**), residual glucose and lactate level (**b**) and glutamine and ammonia levels (**c**)

cultures, respectively. Nevertheless, this level was reached at different times post infection.

Nine harvests were collected when perfusion was used during the virus replication phase. For the repeated-batch culture, only two harvests were obtained. The specific productivity was equal to 1.6 and 0.03 infectious virus particle [IVP] $cell^{-1}$ day^{-1} for the perfused and repeated-batch cultures, respectively. Therefore, using perfusion during the virus replication phase resulted in a higher virus yield when compared to repeated batch. Thus, perfusion was selected as the optimal culture mode for the virus production step.

Figure 3b indicates that glucose level was not limiting during perfused and repeated-batch cultures. Lactate levels observed during cell growth phase were comparable for both cultures. Nevertheless, a higher level of lactate was noticed by the end of the perfused culture. At day 14, lactate levels were equal to 13.5 and 10 mM for the perfused and repeated-batch cultures, respectively.

Regarding glutamine and ammonia levels, no glutamine limitation was observed for both cultures. Average ammonia level was around 3 mM during the cell growth phase. However, residual glutamine level decreased significantly after cell infection when the culture was conducted either in perfusion or repeated-batch culture mode. During this phase, a lower amount of ammonia was produced by the cells, for both cultures.

In an attempt to increase the productivity, we carried out a culture where a lower perfusion rate (0.1 V/day) was applied during the virus production step (Fig. 4). Comparable levels of cell density were obtained during both cultures (Fig. 4a). In addition, similar average specific growth rate, around 0.026 h^{-1}, was observed. Cells were infected at day 7, at an

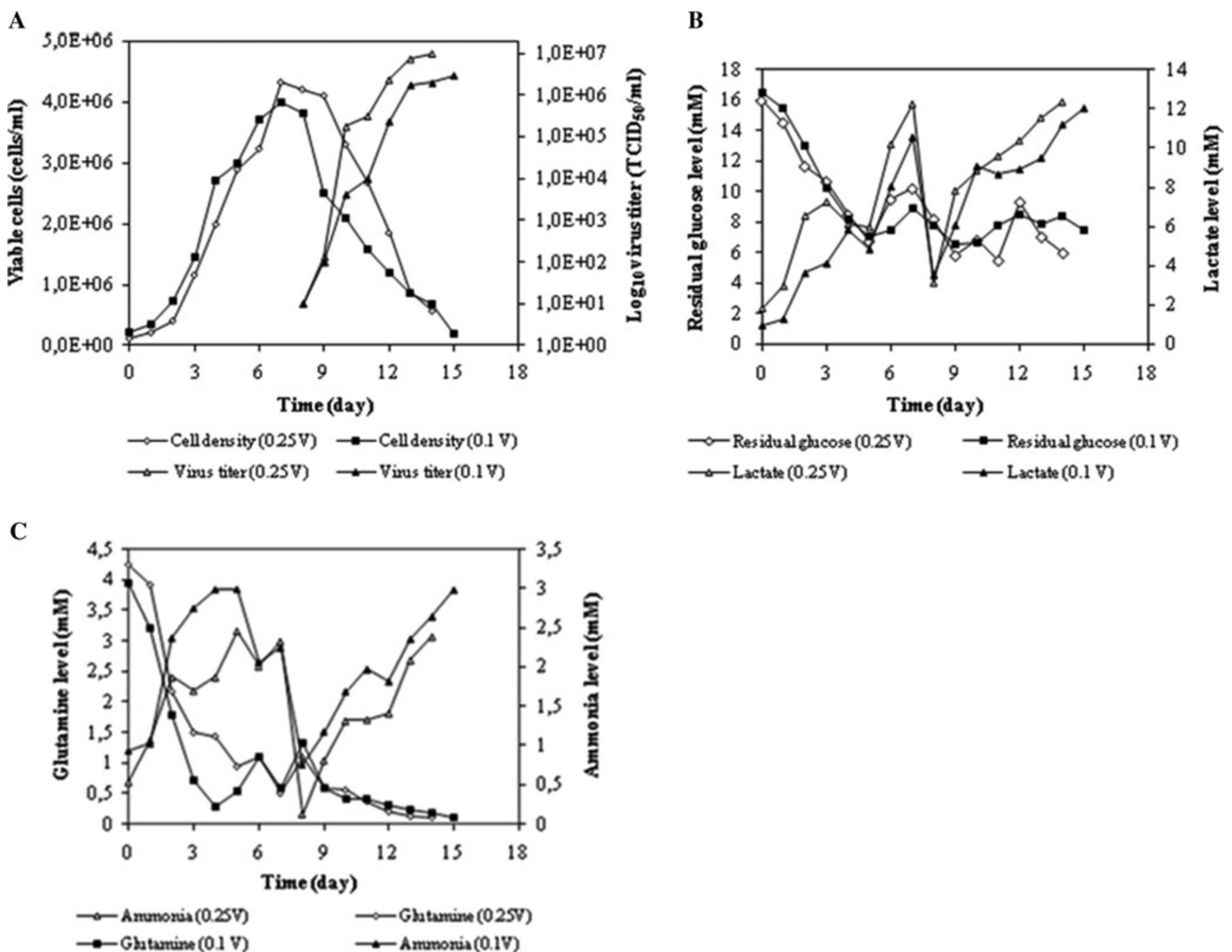

Fig. 4 Effect of the perfusion rate on measles virus production in MRC-5 cells. Cells were grown on 3 g/l Cytodex 1, in a 7-l bioreactor. During cell growth phase, cells were first grown in batch mode then in recirculation mode. Virus production was carried out in M199 at two perfusion rates (0.25 and 0.1 V/day). Cells were infected at day 7 at an MOI of 0.005. Time course of cell density and measles virus titer (**a**), residual glucose and lactate level (**b**) and glutamine and ammonia levels (**c**)

MOI of 0.005, as previously described. After cell infection, the culture was conducted in perfusion at a rate of 0.1 V/day.

Time course of virus titer shows that we obtained a slightly lower titer when compared to the culture perfused at 0.25 V/day. Nevertheless, in this case the virus replication phase was 1 day longer than the 0.25 V/day culture, resulting in a further harvest.

However, the comparison of the specific productivity indicates that the highest specific productivity was reached for the 0.25 V/day culture; its level was 148-fold higher when compared to the 0.1 V/day perfused culture. We achieved a specific productivity of 1.6 IVP $cell^{-1}$ day^{-1} for the highest perfusion rate culture.

Regarding substrate (glucose and glutamine) and metabolite (lactate and ammonia) profiles, glucose level was not limiting during the whole duration of the culture, for both conditions (Fig. 4b). An increase of lactate was observed as glucose was consumed. Similar profiles for glutamine and ammonia are shown in Fig. 4c, although a drastic decrease of glutamine level was observed during the virus production step, as previously shown in Fig. 3c.

Stability testing

Stability data of the vaccine using various stabilizers are shown in Fig. 5. Residual infective virus was highly dependent on the stabilizer used and the storage temperature. The storage stability was the best for the S1-stabilized vaccine. This vaccine exhibited the lowest loss in infectious virus titer under all conditions. At room temperature (around 28 °C), the stability of this vaccine was remarkably higher when compared to the non-stabilized and the S2-stabilized vaccines (Fig. 5). No infectious virus was detected after 3 and 6 days of storage at room temperature for the S2-stabilized and the non-stabilized vaccines, respectively, whereas the S1-stabilized vaccine retains its infectivity to a significant extent after 7 days of storage.

Similar findings were observed at +4 °C and −60 °C temperatures. Nevertheless, the S2-stabilized and the non-stabilized vaccines were more stable under these conditions as compared to the storage at room temperature.

All vaccines showed the highest stability when stored at −60 °C. At this storage temperature, the loss in virus titer was the lowest for all vaccines.

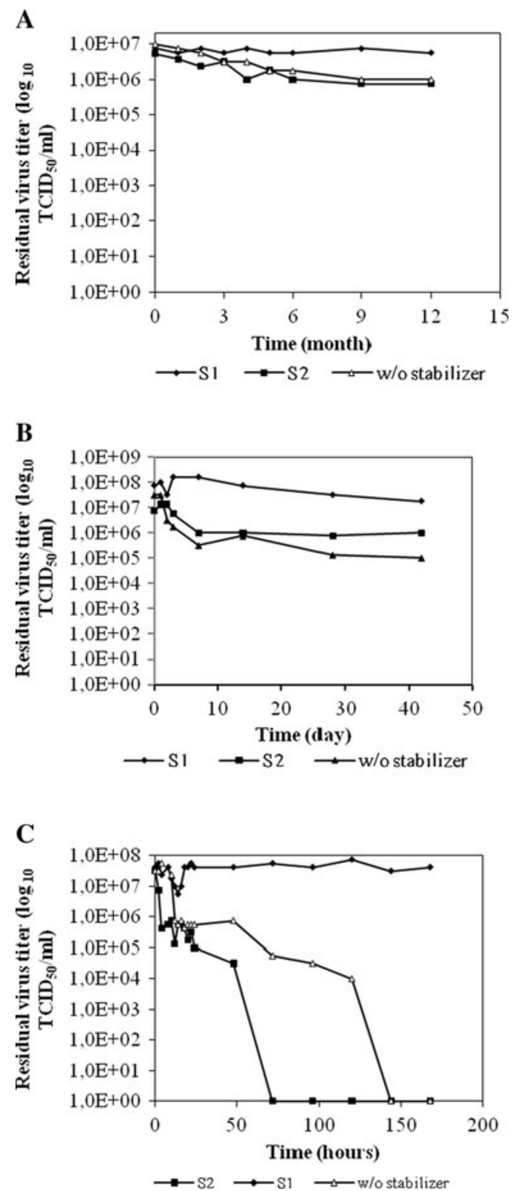

Fig. 5 Stability of measles vaccine using different stabilizers under various storage temperatures. **a** −60 °C, **b** +4 °C and **c** +28 °C

Discussion

In this work, we optimized measles virus production in MRC-5 cells grown on Cytodex 1 microcarriers in a stirred bioreactor. The present study was carried out with the aim of establishing a high yield process for the production of measles vaccine based on the use of microcarrier technology.

The AIK-C strain studied in this work is considered as one of the candidate vaccine strains for the Expanded Programme on Immunization to overcome maternally conferred immunity, resulting in a high sero-conversion rate in young infants at 6 months of age (Tidjani et al. 1989; Nkrumah et al. 1998).

Conditions for AIK-C measles strain replication were first investigated in spinner flask cultures. Kinetics of measles virus production was studied in MRC-5 cells using different culture media: M199, M199+2% FCS, M199+0.2% gelatin and M199+0.5% BSA. Minor improvements of virus titers were observed when M199 was supplemented with FCS, gelatin or BSA. Therefore, M199 was retained for measles virus production in MRC-5 cells. Gelatin and albumin are

common ingredients that are used as a stabilizer during viral vaccines production or in the final product. However, the presence of these products can induce allergic reactions in patients. For instance, for rabies vaccines, residual albumin may provoke systemic hypersensitivity reactions, such as generalized pruritus or urticaria (Lang et al. 1998). In Japan, the use of a measles vaccine that contains gelatin as a stabilizer had provoked an increase of number of cases of anaphylactic/allergic reactions reported to the adverse events monitoring center (Kuno-Sakai and Kimura 2003). Hence, these data support the use M199 without any supplement for measles virus production.

Regulation of glucose level at 1 g/l during the virus production step did not result in a significant improvement of virus titer. These data are in line with those previously obtained in our laboratory for rabies virus production in Vero cells grown on Cytodex 1 in a serum-supplemented medium (Trabelsi et al. 2005); we demonstrated that maintaining glucose level at 1 g/l had increased lactate level without an enhancement of rabies virus titer.

The MOI is a critical factor that has to be carefully determined in a set-up of a viral vaccine cell culture process. MOI can influence virus growth dynamic and virus yield (Genzel et al. 2006). It was demonstrated in numerous studies that in cell infected at high MOIs maximum titers were attained earlier than in cultures infected at lower MOIs (Audsley and Tannock 2005; Maranga et al. 2003). In addition, cell infection at high MOIs increases the amount of defective virus (non-infective) produced by the cells. The optimal MOI is largely dependent on the virus to be produced (whether or not it has a pronounced cytopathic effect); in the case of measles virus, it is preferable to infect the cells at a low MOI. In the present study, we demonstrated that cell infection at an MOI of 0.005,yielded a virus titer comparable to that achieved at an MOI of 0.01.

Cost is a significant factor when considering improvement to vaccination programs in developing countries. One way to reduce vaccine cost is to lower the cost of goods, particularly the amount of serum used during the production process. For this purpose, we studied the effect of diluting of MEM+5% FCS with PBS, on the performance of MRC-5 cell growth on Cytodex 1 in a stirred bioreactor. We showed that medium dilution with 25% PBS resulted in a slightly lower cell density level compared to the non-diluted medium. However, a dramatic decrease of cell density was observed when MEM +5% FCS was diluted with 50% PBS. Therefore, MEM+5% FCS diluted with 25% PBS can be used as a growth medium without a significant loss of cell density. Hence, it appears that the reduction of the serum level from 5% to 3.75% was not deleterious for cell growth.

In this study, we also demonstrated that the use of perfusion as a culture mode during the virus production step, was more efficient than the repeated-batch culture.

Another way to reduce vaccine costs is to generate vaccines with improved stability; vaccines that do not require a cold chain, for example, should be cheaper to deliver and store. Furthermore, a loss in activity of the vaccine under suboptimal storage conditions is thought to contribute significantly to less effective vaccination programs (Brandau et al. 2003). In this work, we demonstrated that measles vaccine that contains sucrose and magnesium as excipients showed a high stability at all the temperatures (28 °C, +4 °C, −60 °C). This vaccine retained its infectivity without a significant loss after storage at 28 °C for 7 days. By contrast, the non-stabilized and the S2-stabilized vaccines exhibited a significant lost of infectious virus when stored at 28 °C. Sugars such as sucrose, lactose and sorbitol are commonly used as stabilizers of measles virus (Ohtake et al. 2010; Kissman et al. 2008). They were reported to act on virus aggregation; however, their effect is depending on the concentration used. Kissman et al. (2008) showed that lactose at 10% inhibited measles virus aggregation at 98% whereas at a concentration of 20%, the percentage of inhibition of aggregation was equal to 69%. Similar data were reported for other sugars and components such as amino acids. Divalents ions such as Ca^{2+}, Zn^{2+} and Mg^{2+} are frequently protein-associated and they have been reported to improve the storage stability of a variety of viruses. In the case of measles virus, divalent cations may serve to interact with the membrane lipids as well as the membrane proteins, thereby helping to maintain the integrity of virus structure (Shirley et al. 1981; Turnbull et al. 2000; Erk et al. 2003).

In conclusion, we developed in this study a high-yield process for the production of measles virus in MRC-5 cells; this process is easily amenable to scale up. We also identified a stabilizer that led to a significant retention of virus infectivity when the vaccine was stored at 28 °C, +4° and −60 °C.

References

Audsley JM, Tannock GA (2005) The growth of attenuated influenza vaccine donor strains in continuous cell lines. J Virol Methods 2:187–193

Aunins JG, Bader B, Caola A, Griffiths J, Katz M, Licari P, Ram K, Ranucci CS, Zhou W (2003) Fluid mechanisms, cell distribution and environment in cellcube bioreactors. Biotechnol Prog 19:2–8

Aunins JG (2000) Viral vaccine production in cell culture. In: Spier ER (ed) Encyclopedia of cell technology. Wiley, New York, pp 1183–1207

Bautista-Lopez N, Ward BJ, Mills E, McCornick D, Martel N, Ratnam S (2000) Development and durability of measles antigen-specific lymphoproliferative response after MMR vaccination. Vaccine 18:1393–1401

Brandau DT, Jones LS, Wiethoff CM, Rexroad J, Middaugh CR (2003) Thermal stability of vaccines. J Pharm Sci 92:218–231

Butler M (1987) Growth limitations on microcarriers. Adv Biochem Eng Biotechnol 34:57–84

Cutts FT, Clements CJ, Bennett JV (1997) Alternative routes of measles immunization: a review. Biologicals 25:323–338

Erk I, Huet J, Duarte M, Duquerroy S, Rey F, Cohen J, Lepault J (2003) A zinc ion controls assembly and stability of the major capsid protein of rotavirus. J Virol 6:3535–3601

Genzel Y, Fischer M, Reichl U (2006) Serum-free influenza virus production avoiding washing steps and medium exchange in large-scale microcarrier culture. Vaccine 24:3261–3272

Griffin DE, Pan CH (2009) Measles: old vaccines, new vaccines. In: Griffin DE, Oldstone MBA (eds) Measles—Pathogenesis and control. Springer, Berlin, pp 191–212

Griffin DE, Bellini WJ (2001) Measles virus. In: Knipe DM, Howley PM (eds) Fields virology, 4th edn. Lippincott Williams & Wilkins, Philadelphia, pp 1267–1312

Griffin DE (1995) Immune response during measles virus infection. Curr Trop Microbiol Immunol 191:117–134

Lang J, Cetre JC, Picot N, Lanta M, Briantais P, Vital S, Le Mener V, Lutsch C, Rotivel Y (1998) Immunogenicity and safety in adults of a new chromatographically purified Vero-cell rabies vaccine (CPRV): a randomized, double-blind trial with purified Vero-cell rabies vaccine (PVRV). Biologicals 4:299–308

Kissman J, Salvador FA, Rudolph A, Braun C, Cape SP, Sievers RE, Federspiel MJ, Joshi SB, Middaugh CR (2008) Stabilization of measles virus for vaccine formulation. Hum Vaccines 4:350–359

Kuno-Sakai H, Kimura M (2003) Removal of gelatin from live vaccines and DTaP—an ultimate solution for vaccine-related gelatin allergy. Biologicals 4:245–249

Maranga L, Brazão TF, Carrondo MJ (2003) Virus-like particle production at low multiplicities of infection with the baculovirus insect cell system. Biotechnol Bioeng 2:245–253

Moss WJ, Griffin DE (2006) Global measles elimination. Nat Rev Microbiol 12:900–908

Montagnon B, Vincent-Falquet JC, Fanget B (1984) Thousand liter scale microcarrier culture of Vero cells for killed polio virus vaccine. Dev Biol Stand 55:37–42

Nechaeva EA, Kashentseva EA, Agafonov AP, Varaksin NA, Ryabicheva TG, Konstantinov AP, Bondarenko VN, Kolokoltsova TD, Kits IV, Senkina TY, Zhilina NV (1998) New form of the live measles vaccine for oral administration. In: Merten OW, Perrin P, Griffiths B (eds) New developments and new applications in animal cell technology. Kluwer Academic Publishing, Dordrecht, pp 573–575

Nkrumah FK, Osei-Kwasi M, Dunyo SK, Koram KA, Afari EA (1998) Comparison of AIK-C measles vaccine in infants at 6 months with Schwarz vaccine at 9 months: a randomized controlled trial in Ghana. WHO Bull 76:353–359

Ohtake S, Martin RA, Yee L, Chen D, Kristensen DD (2010) Heat stable measles vaccine produced by spray drying. Vaccine 28:1275–1284

Radlett PJ (1987) The use of BHK suspension cells for the production of foot and mouth disease vaccines. Adv Biochem Eng Biotechnol 34:129–146

Shirley JA, Beards GM, Thouless ME, Flewett TH (1981) The influence of divalent cations on the stability of human rotavirus. Arch Virol 1:1–9

Sidorenko ES, Dorofeeva LV, Kaptsova TI, Steinberg LL, Zasorina IN, Sinitsyna OA, Boriskin YS (1989) Experimental-scale measles and mumps vaccine production on microcarrier-grown cells. Vaccine 7:554–556

Tidjani O, Grunitsky B, Guerin N, Levy-Bruhk D, Lecam N, Xuereff C, Tatagen K (1989) Serological effects of Edmonston-Zagreb, Schwarz and AIK-C measles vaccine strains given at ages 4–5 or 8–10 months. Lancet 2:1357–1360

Turnbull AE, Skulimowski A, Smythe JA, Alexander IE (2000) Adenoassociated virus vectors show variable dependence on divalent cations for thermostability: implications for purification and handling. Hum Gene Therapy 4:629–635

Trabelsi K, Rourou S, Loukil H, Majoul S, Kallel H (2006) Optimization of virus yield as a strategy to improve rabies vaccine production by Vero cells in a bioreactor. J Biotechnol 121:261–271

Trabelsi K, Rourou S, Loukil H, Majoul S, Kallel H (2005) Comparison of various culture modes for the production of rabies virus by Vero cells grown on microcarriers in a 2-l bioreactor. Enzyme Microb Biotechnol 36:514–519

Tree JA, Richardson C, Fooks AR, Clegg JC, Looby D (2001) Comparison of large-scale mammalian cell culture systems with egg culture for the production of influenza virus A vaccine strains. Vaccine 25–26:3444–3450

Warnock JN, Al-Rubeai M (2006) Bioreactor systems for the production of biopharmaceuticals from animal cells. Biotechnol Appl Biochem 45:1–12

Wolfson LJ, Strebel PM, Gacic-Dobo M, Hockstra EJ, McFarland JW, Hersh BS (2007) Has the 2005 measles mortality reduction goal been achieved? A natural history modelling study. Lancet 369:191–200