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nederlands vaccin instituut

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Evaluation of IPV production on Vero cells on a 1500 liter scale

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1 Summary

An evaluation of production parameters, in-process and release data on the IPV monovalent production process on Vero cells as in use at the NVI is performed.
The production process of IPV monovalent bulk on Vero cells on 1500 liter scale is considered under control.

2 Introduction

2.1 Purpose and Scope

This evaluation is made in order to present an up-to-date overview of the important parameters of the current IPV monovalent production process. Furthermore, with this evaluation the production process of IPV on Vero cells on 1500 Liter scale is assessed for validity with respect to process capability and product quality.

The evaluation is performed on data from release tests and in-process data of all batches monovalent bulk, produced with the Vero cell line on 1500 liter that were produced before August 14th 2007. The first batch included in this evaluation is PV05-311, production date November 17th 2005 and the last batch included is PV07-217, production date 14th of August 2007. During the period mentioned a total of 25 batches of monovalent polio were initiated, consisting of the following types: 6 batches of polio type 1, 5 batches of polio type 2 and 14 batches of polio type 3.

2.2 History

The production process for IPV was initially developed at the predecessors of the NVI - the RIV. The process has been continuously improved since then to keep up with current standards.
In recent years 2 major process changes have been made:
The first change is that Vero cells are now being used in stead of monkey kidney cells. This change was made to decrease the risk of viral contamination and decrease the amount of animals needed for production.
In the second change, the resin used in the Ion exchange chromatography is changed from DEAE sephadex A50 to DEAE sepharose Fast Flow. For this change a viral removal validation (see also Module 3.2.A.2 of the registration file) and a validation of repeated use of DEAE sepharose (see also Module 3.2.S.2.5 of the registration file) has been performed

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2.3 Process description

The process flow of the Polio production is represented in paragraph 2.4. A brief summary of the production process of IPV on Vero cells on a 1500 liter scale (in building U4 at the NVI) is given in this chapter. Prior to the evaluation of each production step, a more detailed description of that production step is given.

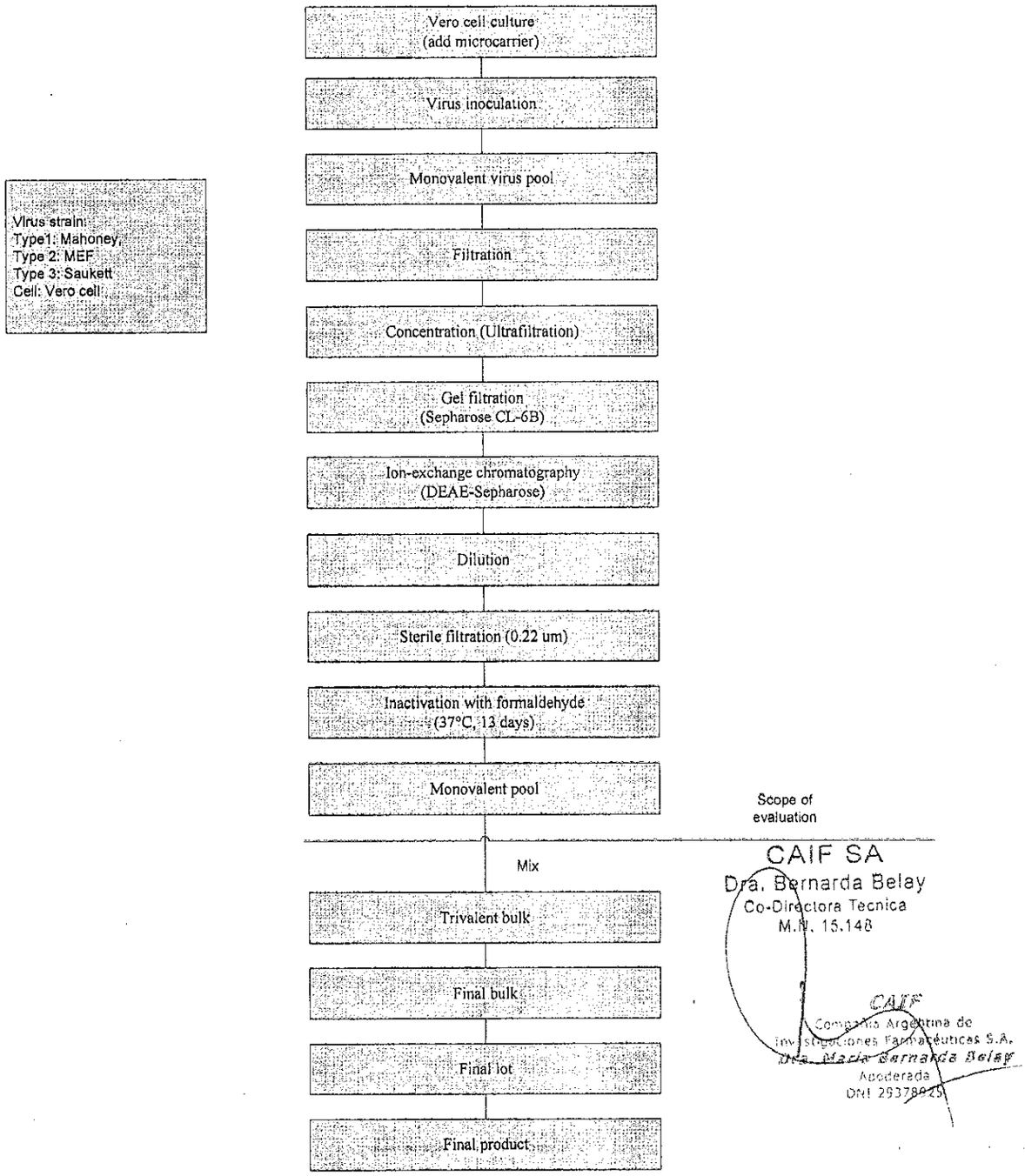
IPV production starts with the cultivation of host cells (Vero cell line) in 3 consecutive culture steps (passages). The Vero cells are grown on Cytodex micro carriers in bioreactors on 40 liter scale. The cells are treated with Trypsin in order to allow passage of the Vero cells. Before the 3rd passage, the Vero cells are split and transferred into two main bioreactors (stainless steel 750 liter bioreactors). In these bioreactors the third passage takes place followed by a medium exchange.

The Vero cells are inoculated with the poliovirus (Mahoney for type 1, MEF for type 2 and Saukett for type 3) and after proliferation the virus is harvested and purified using the following steps: clarification, concentration (ultra filtration), size exclusion chromatography and ion exchange chromatography.

Finally, the purified poliovirus pool is filtered and thereafter inactivated with formaldehyde resulting in a monovalent bulk.

For formulating of the trivalent bulk IPV, at least three monovalent bulks (at least one of each type poliovirus) are mixed in a predefined ratio. The formulation of trivalent bulk as well as final bulk is out of the scope of this evaluation.

2.4 Process flow



2.5 Method

This process evaluation consists of an assessment of release and in-process data. The evaluation of the process and release related data are used as objective parameters for capability and robustness of the production process.

2.5.1 Evaluation of in process and release data of monovalent batches produced

The intention of this study is to perform an adequate evaluation of the IPV production process with respect to consistency, product quality and process capability.
For relevant in-process and release data the mean and the standard deviations were calculated. The mean plus 2 times the standard deviation (mean + 2SD) and the mean minus 2 times the standard deviation (mean - 2SD) were calculated. Results obtained were evaluated against these mean + or – 2SD. The production of IPV monovalent bulk is a biological process. The RSD (relative standard deviation, calculated by deviding the standard deviation by the mean) as observed for some parameters is higher than expected for a purely chemical production process. During this evaluation the higher variability found for biological processes is taken into account.

The same Vero cell cultivation steps are used for the different types of monovalent polio. Therefore, the results obtained during these process steps can be included in one evaluation (no separate evaluation is necessary per type). In total results of 25 batches were included in the evaluation. During the production process 5 batches were aborted. For each process step or parameter all batches that reached that process step are part of the evaluation. For every parameter or process step the number of batches available is given (n).

After the cultivation of the Vero cells, the cells are inoculated with one of the different types of polio. Although the production steps and manufacturing conditions are identical, the relevant results are evaluated per polio type. This approach was chosen, because it can not be excluded that the same conditions will have different effects on the different types of polio.

In chapter 3 the results of the data evaluation are summarized and discussed.

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3 Evaluation of release and in process data

For each step in the production process, relevant data is summarized and the mean and the standard deviation is calculated. Per parameter the mean + 2SD and the mean - 2SD is calculated. These parameters are not intended as limits but are used for evaluation purposes only. Where relevant, the number of batches outside this range is shown. Data is summarized in tables followed by an evaluation. At the end of the paragraph cross references between different parameters are discussed.

3.1 Cell culture

3.1.1 first passage of cell growth

The Vero cells of the working cell bank (MWCB) are inoculated and incubated for 6 (5-7) days in a glass bioreactor with 15 liters of medium and micro carriers (Cytodex) as described in module 3.2.S.2.3 of the registration file. The amount of medium is increased till an amount of 40 liter is reached. The pH is controlled with sodium bicarbonate and kept on 7.2. Glucose is added if the glucose concentration is below the set point of 5.5 mM.

For this evaluation all 25 batches have been used. (n=25)

Table 3.1.1a: Number of vital cells (per ml) used for inoculation

Mean	7.89E+08
Standard Deviation	2.65E+08
Mean - 2SD	2.58E+08
Mean + 2SD	1.32E+09
Number below mean - 2SD	2
Number above mean + 2SD	0

The standard deviation is relatively high. This is to be expected, since freezing and thawing of working cell stock can have influence on the vitality of cells. Since this high variation in the number of vital cells is not seen anymore in the Vero cells by the end of the first passage (table 3.1.1c) the results obtained are considered acceptable.

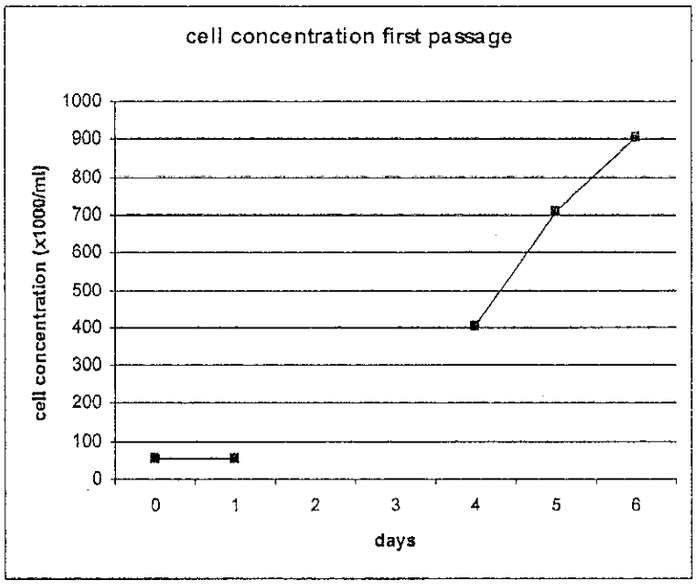
Table 3.1.1b: Micro Carrier concentration (g/l)

Mean	3.36
Standard Deviation	0.10
Mean - 2SD	3.16
Mean + 2SD	3.56
Number below mean - 2SD	0
Number above mean + 2SD	2

The RSD is about 3%, this is an acceptable value.

Table 3.1.1c: Cell concentration (x 1000 cells / ml)

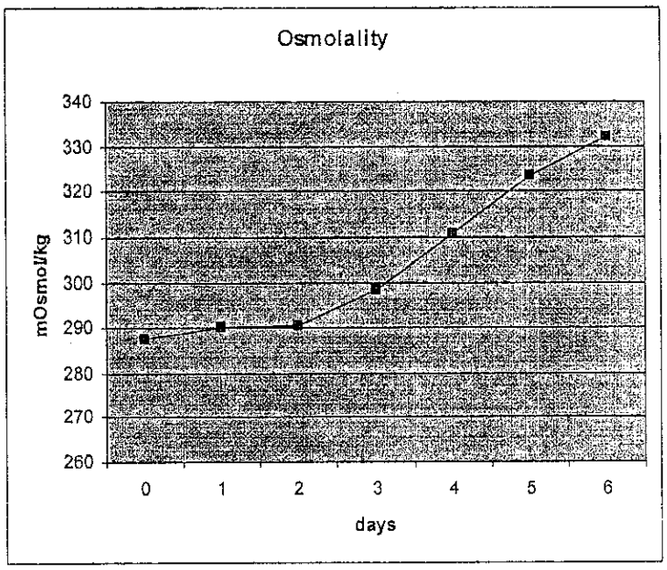
	Day 0	Day 1	Day 4	Day 5	Day 6
Mean	52	52	402	707	906
Standard Deviation	13	9	54	69	89
Mean - 2SD	25	33	294	570	728
Mean + 2SD	78	71	510	845	1084
Number below mean - 2SD	0	0	0	0	0
Number above mean + 2SD	2	0	0	1	1



The results show no increase in cell concentration the first day. This can be due to the fact that the cells need some time to adapt to the new environment and have to become attached to the micro carriers (lag-phase). The growth curve shows an exponential increase during the following days (log-phase). There are no signs of reaching a plateau. Transfer of the cells to the second passage takes place in the log-phase.
The standard deviation is relatively low (less than 10% in the final days) compared to the parameter measured and the analytical method (manual count) used. This shows that the process of cell cultivation is under control.

Table 3.1.1d: Osmolality (mOsmol/kg)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Mean	288	290	290	298	311	323	332
Standard Deviation	7	6	6	8	9	8	8
Mean - 2SD	274	278	278	282	293	307	316
Mean + 2SD	302	302	302	314	329	339	348
Number below mean - 2SD	0	0	0	0	0	0	0
Number above mean + 2SD	0	0	0	0	0	0	0



The osmolality is indicative for the concentration of nutrients and ions present in the culture medium. An increase in osmolality is seen. The pH drops because of the excretion of waste products. To compensate for this drop in pH, sodium bicarbonate is added and this results in an increase of the osmolality.
The standard deviation is very low, this indicates that the increase in osmolality in the culture medium is reproducible. In addition, it indirectly shows that the cell growth (and excretion of waste products) is reproducible.

Table 3.1.1e: Glucose concentration (mmol/l)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Mean	8.3	8.3	7.5	6.2	3.0	2.1	3.0
Standard Deviation	0.7	0.7	0.7	0.7	0.7	0.9	0.7
Mean - 2SD	7.0	6.9	6.0	4.9	1.6	0.3	1.6
Mean + 2SD	9.7	9.6	9.0	7.5	4.4	3.8	4.4
Number below mean - 2SD	0	0	0	0	0	0	0
Number above mean + 2SD	0	0	0	0	0	0	0

Table 3.1.1f: Extra glucose (ml)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Mean	0	0	0	1	63	106	25
Standard Deviation	0	0	0	2	19	31	45

Since the RSD is high, the mean + 2SD and the mean - 2SD for extra glucose does not add additional value and can be discarded.

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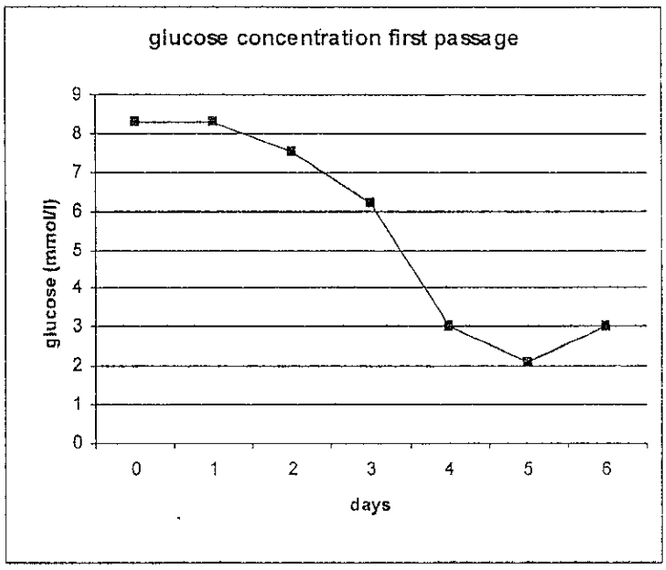

For the glucose concentration a set point of 5.5 mmol/l is used. Below this set point glucose is added. During the cultivation step, glucose will be needed as an energy source of the cells, the increase in glucose consumption as the cells proliferate results in either a drop in glucose concentration or an increase in the amount of glucose added.
The largest amount of glucose is added at the end of the cultivation step. This is expected since at the end of this step the amount of cells also reaches its highest concentration.

Table 3.1.1g: Extra sodium bicarbonate (ml)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Mean	0	0	2	123	393	511	603
Standard Deviation	0	0	8	63	98	85	131

Since the RSD is high, the mean + 2SD and the mean - 2SD for extra sodium bicarbonate does not add additional value and can be discarded.

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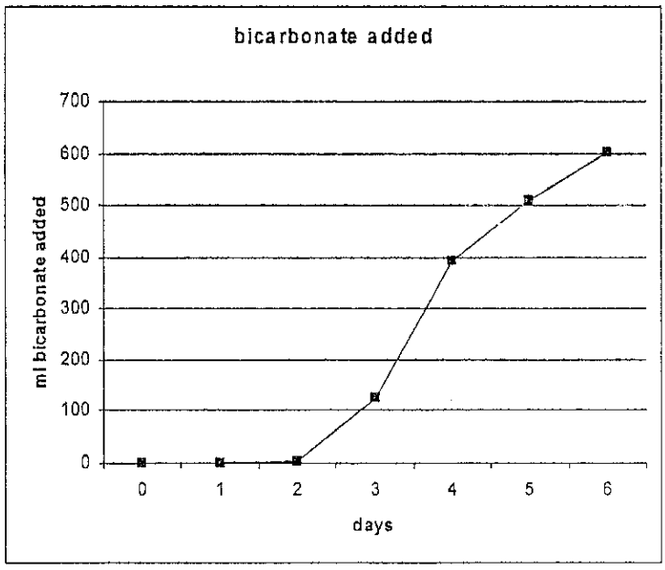

The growth curve (number of cells present) is reflected in the amount of sodium bicarbonate needed for regulation of the pH at a constant level (pH = 7.2) in combination with in-line pH determination. This is expected as the pH is influenced by the cell excretions. The mean + 2SD and the mean - 2SD for this parameter do not add additional value and can be discarded.
The amount of sodium bicarbonate to be added depends on multiple factors, for instance the amount of cells, cell growth, the phase of the cells and the amount of CO_2. The many factors and the variability of each factor may lead to high variation between batches. Therefore, although the standard deviation seems relatively high, this is an acceptable variation for this process step. Furthermore, all other parameters are under control or kept constant.

Table 3.1.1h: Total number of cell divisions ($2\log C_t/C_0$)

Mean	4.2
Standard Deviation	0.4
Mean - 2SD	3.4
Mean + 2SD	5.0
Number below mean - 2SD	2
Number above mean + 2SD	0

The standard deviation with regard to the mean number of cell divisions is relatively low taken in respect the parameter measured. This shows that this process step is under control.

Conclusion
It can be concluded that the first culture step is under control; the growth curve of the cells show the normal phases of the cell-culture. The same curve can be seen in the consumption of glucose and the need for addition of Sodium Bicarbonate. Also the number of cell division is representative for the different batches (25 in total).

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3.1.2 Data analysis second passage

The Vero cells are being trypsinized using trypsine in citrate buffer in an external vessel. The cells are transferred to the 40 L glass bioreactor filled with fresh medium (day 0). The second growth phase takes place for 5 – 7 days in medium and micro carriers (Cytodex) as described in module 3.2.S.2.2 of the registration file. The medium is recirculated during this cultivation step.

For this evaluation all 25 batches have been used

Table 3.1.2a Recovery of Vero cells after trypsinisation (%)

Mean	84.5
Standard Deviation	12.8
Mean - 2SD	58.8
Mean + 2SD	110.1
Number below mean - 2SD	1
Number above mean + 2SD	1

During the trypsinisation, the Vero cells are separated from the micro carriers and both Vero cells and micro carriers are added to new medium with extra micro carriers. The recovery of Vero cells in the new medium with new micro carriers is an important parameter. The recovery is about 85 % which is an acceptable high recovery. For one batch a recovery above 100% was found. This is not a logical value since no cells were added and should therefore be seen as an outlier or can be due to the variance in the determination of the amount of cells. The standard deviation even with the result above the 100% recovery included is relatively low (about 15 %) compared to the parameter measured. This shows that the process regarding the recovery of Vero cells after trypsinization is under control.

Table 3.1.2b Micro carrier concentration (g / l)

Mean	3.74
Standard Deviation	0.09
Mean - 2SD	3.56
Mean + 2SD	3.92
Number below mean - 2SD	0
Number above mean + 2SD	1

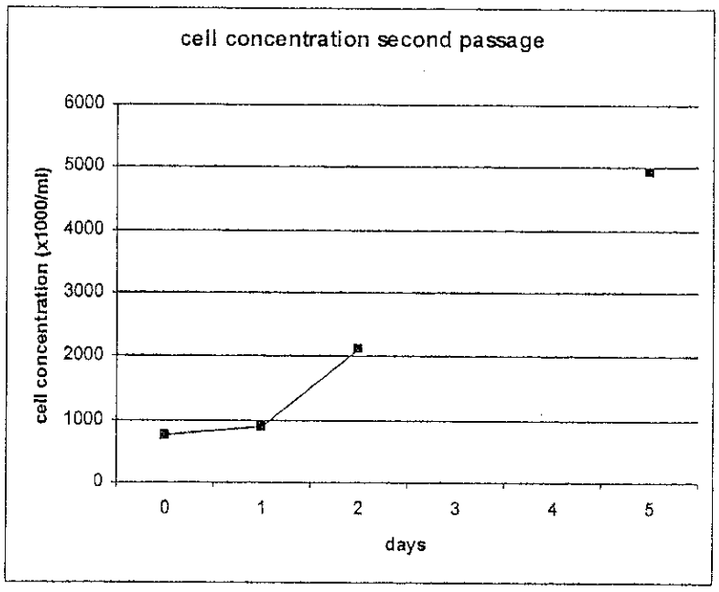
The RSD is about 2%, this is an acceptable value.

Table 3.1.2c Cell concentration (x 1000 cells / ml)

	Day 0	Day 1	Day 2	Day 5	Day 6
Mean	763	873	2123	4938	4455*
Standard Deviation	105	104	334	694	N/A*
Mean - 2SD	553	665	1454	3550	N/A*
Mean + 2SD	973	1081	2791	6327	N/A*
Number below mean - 2SD	1	1	0	1	N/A*
Number above mean + 2SD	1	0	0	0	N/A*

*this value is only measured for one batch; all other batches have been transferred before day 6 (n=1)
N/A = not applicable

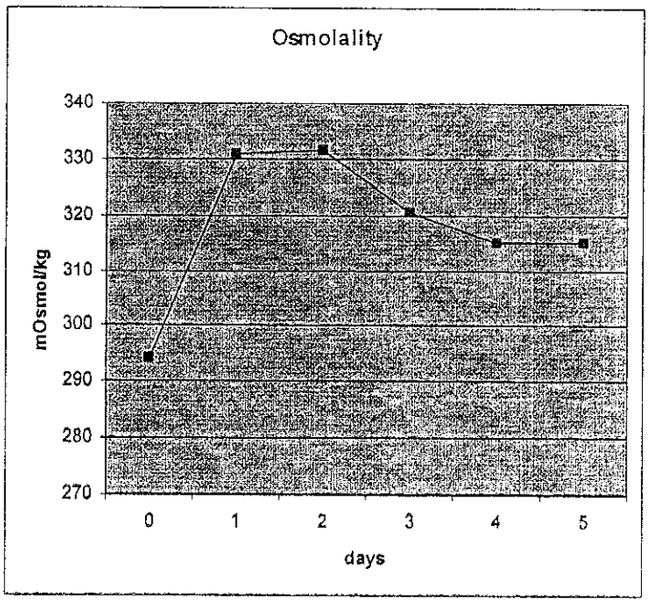
One batch is measured on day 4 in stead of day 5. This day 4 value is excluded from the calculations of the day 5 results. One batch is cultivated 1 day longer, so an extra measurement was performed on day 6.



A minimal increase in cell concentration was seen the first day; most probably the cells need some time to adapt to the new environment and have to become attached to the micro carriers (lag-phase), equivalent with the first passage. The growth curve seems to show an exponential increase during day 2-5 (log-phase). Transfer of the cells to the third passage seems to take place in the log-phase. The standard deviation is relatively low (about 14 %) compared to the parameter measured and the analytical method (manual count) used. This shows that the process regarding the cell concentration during the second passage is under control.

Table 3.1.2d Osmolality (mOsmol/kg)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Mean	294	331	332	320	315	315
Standard Deviation	10	10	9	6	6	6
Mean - 2SD	273	311	314	308	303	303
Mean + 2SD	315	351	350	332	327	327
Number below mean - 2SD	0	1	1	0	0	0
Number above mean + 2SD	1	1	1	1	1	1



The osmolality is indicative for the concentration of nutrients and ions present in the culture medium. A slight increase in osmolality is seen in the first days. The increase can be explained by the fact that the concentration sodium bicarbonate becomes higher during the culture phase, since this is used for keeping the pH at a constant level. A decrease of osmolality is seen during day 3-5. This seems to be an unusual result. However, on the first day recirculation is started. During recirculation the fluids of the bioreactor are recirculated through the other vessels with an increasing flow. Because of that the homogeneity between the bioreactor and the vessels is graduately increased. At the end of the recirculation there is still no complete homogeneity. Therefore during the entire second passage the amounts of fluids, in combination with the increasing flow, are sufficient to keep the pH constant without addition of sodium bicarbonate. The pattern seen for addition of sodium bicarbonate is equivalent with the pattern of osmolality, taking into account the fact that osmolality is a cumulative parameter and sodium bicarbonate is not.

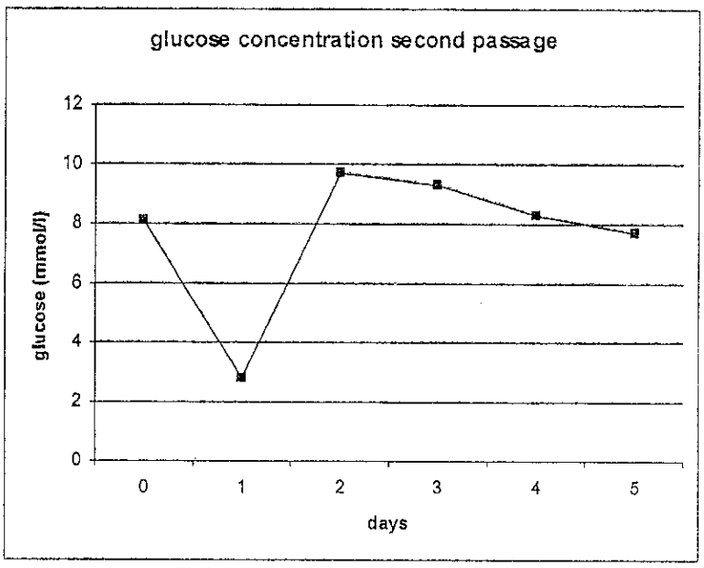
Table 3.1.2e Glucose concentration (mmol/l)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Mean	8.1	2.8	9.7	9.3	8.3	7.7
Standard Deviation	0.8	1.1	2.0	1.0	1.0	1.0
Mean - 2SD	6.4	0.6	5.7	7.2	6.4	5.6
Mean + 2SD	9.8	5.0	13.8	11.3	10.3	9.7
Number below mean - 2SD	0	1	1	1	0	0
Number above mean + 2SD	1	1	1	1	0	1

Table 3.1.2f Extra glucose (ml)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Mean	0	97	0	0	0	0
Standard Deviation	0	54	0	0	0	0

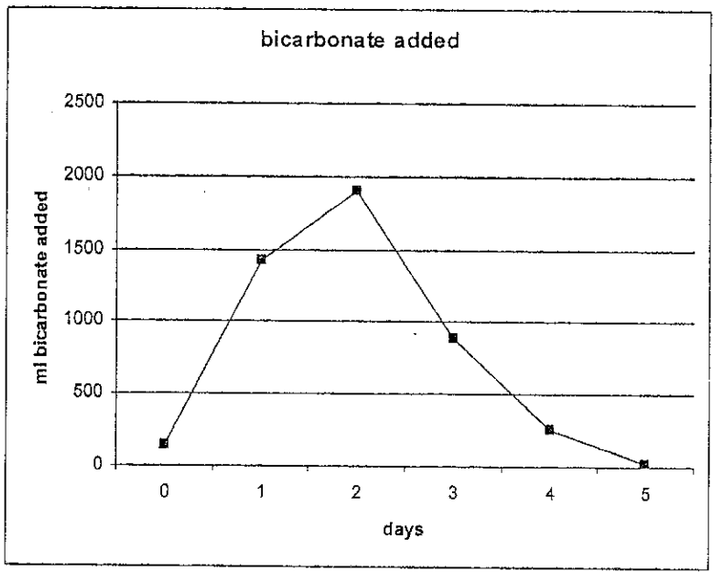
Since the RSD is high, the mean + 2SD and the mean - 2SD for extra glucose does not add additional value and can be discarded.



The glucose concentration shows a drop on day 1, the same day that glucose is added in all batches produced and 1 liter of glucose is added in the second recirculation vessel. The concentration of glucose slightly decreases over days 3 to 5, due to glucose consumption of the cells.
After this first addition of glucose on day one, no glucose is added on the following days. Even then the glucose level does not drop below the glucose level of 7,7 mmol/l. This indicates that the glucose concentration is sufficient.
The consumption of glucose during the complete second cultivation step is reproducible.

Table 3.1.2g Extra sodium bicarbonate (l)

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Mean	145	1421	1907	886	252	25
Standard Deviation	182	315	434	727	313	73
Mean - 2SD	-219	791	1039	-568	-374	-121
Mean + 2SD	509	2051	2775	2340	878	171
Number below mean - 2SD	0	0	0	0	0	0
Number above mean + 2SD	1	0	1	1	1	1



Until day 2, the growth curve (number of cells present) is reflected in the amount of sodium bicarbonate needed (to keep the pH at a constant level; pH = 7,2). This is expected as the pH is influenced by the cell excretions. However, this pattern is not seen during the second half of this cultivation step, where a decrease in the need for sodium bicarbonate is seen, while the amount of cells is increasing. This can be explained by the fact that the recirculation is started at day 1. See also table 3.1.2d.

The mean + 2SD and the mean - 2SD do not add additional value for this parameter and can be discarded. The amount of sodium bicarbonate to be added is dependent on multiple factors, for instance the amount of cells, cell growth, the phase of the cells and the amount of CO_2. Therefore, although the standard deviation seems relatively high, this is an acceptable variation for this process step. Furthermore, all other parameters are under control or kept constant.

Table 3.1.2h Total number of cell division ($2\log C_t/C_0$)

Mean	2.7
Standard Deviation	0.3
Mean - 2SD	2.1
Mean + 2SD	3.3
Number below mean - 2SD	1
Number above mean + 2SD	1

The standard deviation with regard to the mean is relatively low taken in respect the parameter measured. This shows that this process step is under control.

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Conclusion:
It can be concluded that the second culture step is under control; the growth curve of the cells show the normal phases of the cell-culture, while passage to the third passage seems to take place in the log-phase. The curves as seen in the consumption of glucose and the need for addition of Sodium Bicarbonate follow a different pattern which can be explained by addition of 1 liter of glucose on day 1 to the second recirculation vessel (glucose) and the recirculation (sodium bicarbonate).
The number of cell divisions is representative for the different batches (25 in total).

*3.1.3 Data analysis third passage (n=2*24)*

The Vero cells are being trypsinized using trypsine in citrate buffer. The cells are transferred into two 750 liter bioreactors. All process steps including in-process controls are performed in each bioreactor separately. The third growth phase takes place for 5 – 7 days in medium and micro carriers (Cytodex) as described in module 3.2.S.2.2 of the registration file The pH is controlled with sodium bicarbonate and kept on 7.2. Glucose is added if the glucose concentration is below the set point of 5.5 mM. Vero cells are cultured to a maximum of 15 cell divisions (including the cell division of the first and second passage)
Before start of the 3rd passage 1 batch was aborted, therefore the amount of batches at start of the 3rd passage is 24. During the 3rd passage another batch was aborted resulting in 23 batches left. Each batch is divided into two bioreactors and measures independently. Although the content of both bioreactors originate from the same batch in the second passage and are reunited after virus cultivation for the evaluation each measurement is seen as an independent measurement. Therefore n= 2*24 respectively 2*23.

Table 3.1.3a Recovery of Vero cells after trypsinisation (%; n=2*24)

Mean	65.9
Standard Deviation	26.1
Mean - 2SD	13.7
Mean + 2SD	118.1
Number below mean - 2SD	0
Number above mean + 2SD	2

During the trypsinisation, the Vero cells are separated from the micro carriers and both Vero cells and micro carriers are added to new medium with extra micro carriers. The recovery of Vero cells in the new medium with extra micro carriers is an important parameter. The recovery is about 65 % which is lower then the recovery after the first passage. The standard deviation is relatively high (about 40 %) compared to the parameter measured.

Table 3.1.3b Micro carrier concentration (g / l; n=2*24)

Mean	3.2
Standard Deviation	0.2
Mean - 2SD	2.8
Mean + 2SD	3.6
Number below mean - 2SD	0
Number above mean + 2SD	2

The RSD is below 1%, this is an acceptable value.

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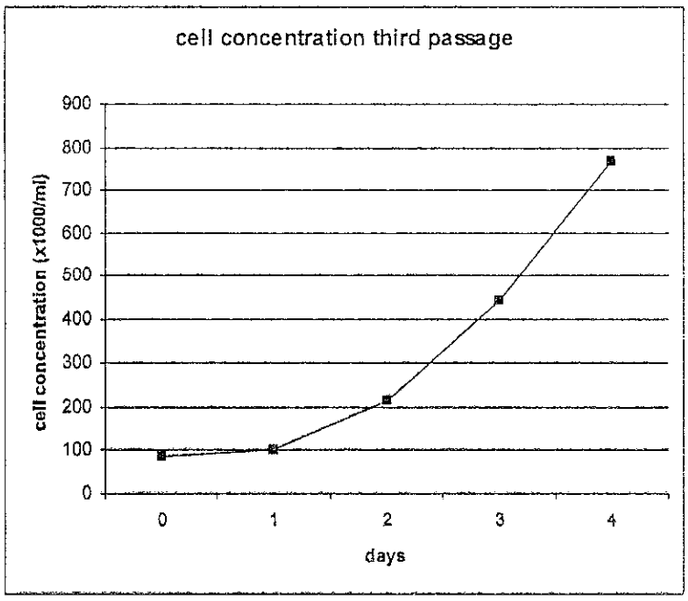
Table 3.1.3c Cell concentration (x 1000 cells / ml)

	Day 0 (n=2*24)	Day 1 (n=2*24)	Day 2 (n=2*24)	Day 3 (n=2*24)	End of culture (n=2*23)
Mean	85	101	215	444	766
Standard Deviation	23	13	33	83	67
Mean - 2SD	39	75	149	279	633
Mean + 2SD	132	128	282	610	899
Number below mean - 2SD	0	0	0	1	1
Number above mean + 2SD	1	2	1	2	0



The results show a minimal increase in cell concentration the first day. This can be due to the fact that the cells need some time to adapt to the new environment and have to become attached to the micro carriers (lag-phase). The growth curve shows an exponential increase during day 2-4 (log-phase). There are no signs of reaching a plateau. Inoculation of the cells using the polio viruses clearly takes place in the log-phase. The standard deviation is relatively low (about 10-20 %) compared to the parameter measured and the analytical method (manual count) used. This shows that the process regarding the cell concentration is under control.

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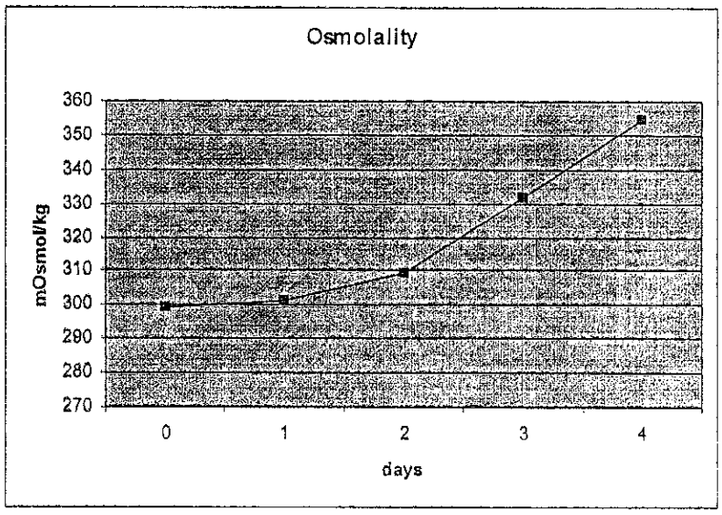
Table 3.1.3d Osmolality (mOsmol/kg)

	Day 0 before inoculation (n=2*24)	Day 0 (n=2*24)	Day 1 (n=2*24)	Day 2 (n= 2*24)	Day 3 (n=2*24)	End of culture (n=2*23)
Mean	298	299	301	309	332	355
Standard Deviation	8	11	10	18	8	13
Mean - 2SD	282	278	281	273	316	329
Mean + 2SD	315	320	321	345	348	381
Number below mean - 2SD	1	3	3	1	1	0
Number above mean + 2SD	1	2	0	2	1	1



The osmolality is indicative for the concentration of nutrients and ions present in the culture medium. The osmolality increases slightly over the period of incubation. The increase can be explained by the fact that the concentration sodium bicarbonate increases during the culture phase, since this is used for keeping the pH at a constant level. The pattern seen for addition of sodium bicarbonate is equivalent with the pattern of osmolality taking into account the fact that osmolality is a cumulative parameter and sodium bicarbonate is not.
The standard deviation is very low; this indicates that the culture medium with respect for the osmolalilty is standardized and reproducible.

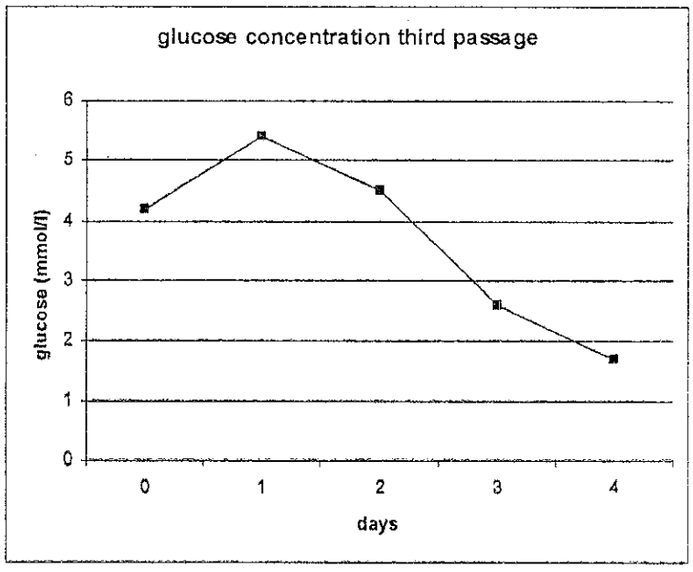
Table 3.1.3e Glucose concentration (mmol/l)

	Day 0 (n=2*24)	Day 1 (n=2*24)	Day 2 (n= 2*24)	Day 3 (n=2*24)	End of culture (n=2*23)
Mean	4.2	5.4	4.5	2.6	1.7
Standard Deviation	0.3	0.3	0.4	0.5	0.7
Mean - 2SD	4.2	5.4	4.5	2.6	1.7
Mean + 2SD	0.3	0.3	0.4	0.5	0.7
Number below mean - 2SD	0	2	1	1	2
Number above mean + 2SD	1	2	0	1	2

Table 3.1.3f Extra glucose (ml)

	Day 0 (n=2*24)	Day 1 (n=2*24)	Day 2 (n= 2*24)	Day 3 (n=2*24)	End of culture (n=2*23)
Mean	931	48	686	2116	0
Standard Deviation	210	147	370	599	0

Since the RSD is high, the mean + 2SD and the mean - 2SD for extra glucose does not add additional value and can be discarded.



For the glucose concentration a set point of 5.5 mmol/l is used. Below this set point glucose is added.

Addition of extra glucose, is seen for day 0, 2 and 3. On day 1 only in some cases glucose is added. On day 4 no glucose is added. This is the day that the medium is changed for virus inoculation. The largest consumption of glucose is expected on the last days, since the cells are still in there log-phase and the cell concentration is at highest. This can be seen by the fact that the concentration is decreasing, even with a high amount of glucose added on day 3.

The glucose consumption is reproducible for this cultivation step.

Table 3.1.3g Extra sodium bicarbonate (l)

	Day 0 (n=2*24)	Day 1 (n=2*24)	Day 2 (n= 2*24)	Day 3 (n=2*24)	End of culture (n=2*23)
Mean	0	0.5	4.1	13.8	14.5
Standard Deviation	0	0.9	2.0	2.4	6.7
Mean - 2SD	0	-1.3	0.1	9.0	1.1
Mean + 2SD	0	2.3	8.1	18.6	27.9
Number below mean - 2SD	0	0	0	1	4
Number above mean + 2SD	0	3	1	2	0

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