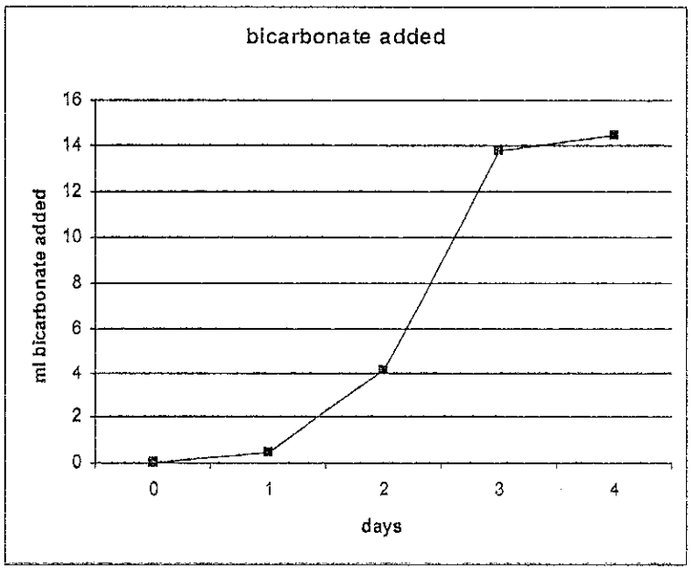

The growth curve (number of cells present) is reflected in the amount of sodium bicarbonate needed (to keep the pH at a constant level; pH = 7.2). This is expected as the pH is influenced by the cell excretions. The mean + 2SD and the mean - 2SD for this parameter do not add additional value and can be discarded. The amount of sodium bicarbonate to be added 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 3.1.3h Number of cell division third passage ($2\log C_t/C_0$) (n=2*23)

Mean	3.2
Standard Deviation	0.4
Mean - 2SD	2.4
Mean + 2SD	4.0
Number below mean - 2SD	0
Number above mean + 2SD	2

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

Table 3.1.3i Total number of cell division during complete culture phase (first, second and third passage) ($2\log C_t/C_0$) (n=46)

Mean	10.0
Standard Deviation	0.8
Mean - 2SD	8.4
Mean + 2SD	11.6
Number below mean - 2SD	1
Number above mean + 2SD	2

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The relative standard deviation with regard to the mean is relatively low taken into account the parameter measured. Furthermore, the process, as is used, is capable to process within the limit of less than 15 cell divisions. This shows that this process step is under control.

Conclusion:
It can be concluded that the third culture step is under control; the growth curve of the cells show the normal phases of the cell-culture, while inoculation takes place in the log-phase. The same curve can be seen in the consumption of glucose and the need / addition of Sodium Bicarbonate. The number of cell division is representative for the different batches.

3.2 Virus inoculation

The medium is changed to medium as described in module 3.2.S.2.3 of the registration file.
The three types of poliomyelitis virus are separately cultured on Vero cells in two 750L bioreactors, this means that one batch of Vero cells produced will only be used for one batch of a specific poliomyelitis virus type. Therefore, the amount of batches for each specific type is much lower than was seen for the evaluation of the culture phase. The n drops to a level were the number below the mean - 2SD and the number above the mean + 2SD do not have a statistical meaning and will therefore not be a part of this evaluation.
During down stream processing 3 batches were aborted. These batches were not tested for D-antigen Therefore, for the volume of virus harvest the total number of batches is 23 and for all parameters after virus harvest the total umber of batches is 20.

Table 3.2a Volume virus harvest

parameter	Type 1 (n=6)	Type 2 (n=5)	Type 3 (n=12)
Mean	1502	1487	1489
Standard Deviation	36	6	6
Mean - 2SD	1429	1475	1477
Mean + 2SD	1575	1499	1501

The volume after virus harvest is about 1450 l and very reproducible for all batches produced. This could be expected since the amount of media started is the same for all batches. Changes are the consequences of addition of glucose and sodium bicarbonate. However, the amount added of these products is very low in comparison to the initial volume.

Table 3.2b D-antigen concentration after harvest (DE/ml)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	104	29	63
Standard Deviation	12	4	8
Mean - 2SD	80	20	47
Mean + 2SD	129	37	80

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There is a clear difference in D-antigen concentration between the different types. This is expected, since different types of poliomyelitis are used and different types of IPV are produced and measured.
The relative standard deviation is as expected (about 20%) with regard to the parameter measured and the method used for analyzing D-antigen.

Conclusion:
The results show that the virus inoculation is under control.

3.3 Clarification

Clarification is performed by filtering the virus harvest over a series of 3 filters with a decreasing pore size (75 µm - 0,45 µm - 0,22 µm).

Table 3.3a D-antigen concentration (DE/ml) after clarification with in-process test

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	104	27	59
Standard Deviation	21	5	9
Mean - 2SD	61	17	41
Mean + 2SD	146	37	77

There is a clear difference in D-antigen concentration between the different types. This is expected since, different types of poliomyelitis are used and different types of IPV are produced and measured.
The standard deviation is as expected (about 20%) with regard to the parameter measured and the method used for analyzing D-antigen.

Conclusion:
The results obtained show that the clarification step is under control.

3.4 Concentration

During concentration the clarified fraction is reduced from 1500 liter to approximately 1 to 2,5 liter of concentrated virus. The virus is concentrated using ultra filter membranes (100.000 Dalton) build in into the ultra filtration system.

Table 3.4a Volume (l)

parameter	Type 1 (n=6)		Type 2 (n=4)		Type 3 (n=10)	
	before	after	before	after	before	after
Mean	1633	1.7	1621	1.7	1604	1.8
Standard Deviation	22	0.1	10	0.0	23	0.1
Mean - 2SD	1589	1.5	1601	1.7	1558	1.6
Mean + 2SD	1677	2.0	1641	1.7	1650	1.9

The volume before filtration is about 1600 liters for all types. The volume is reproducible since the RSD found for this parameter is very low (about 1%). The volume after filtration is about 1.7 liters for all types. Since the volume is controlled during this process step, the high reproducibility found for the volume after concentration was expected.
During this process step an enormous reduction in volume (about 1000 times) is obtained as designed.

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Table 3.4b D-antigen concentration (DE/ml)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	71174	18625	30628
Standard Deviation	13173	4489	9373
Mean - 2SD	44828	9647	11882
Mean + 2SD	97519	27603	49374

There is a clear difference in D-antigen concentration between the different types. This is expected, since different types of poliomyelitis virus are used and different types of IPV are cultured and measured.
The relative standard deviation is as expected (about 20%) with regard to the parameter measured and the method used for analyzing D-antigen

Conclusion:
The concentration step is under control.

3.5 Size exclusion Chromatography

For the first purification step size exclusion chromatography over Sepharose CL-6B is performed. Molecules with a MW of 10^4 - 4 x 10^6 are separated. This step is also used to transfer the virus suspension into a buffer with a low ion concentration, which is necessary for the next purification step. Purification with size exclusion chromatography is performed and monitored by UV detection.

Table 3.5a Initial Volume (l)

Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	1.7	1.7	1.8
Standard Deviation	0.1	0.0	0.1
Mean - 2SD	1.5	1.7	1.6
Mean + 2SD	2.0	1.7	1.9

The initial column volume is the volume as was obtained from the concentration step minus the samples taken (see table 3.4a) and is about 1.7 l. It is not expected that little changes in initial volumes on the column will result in a different chromatographic pattern.

Table 3.5b Volume fraction 3.1 (l)

Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	9.9	10.3	10.2
Standard Deviation	0.8	0.6	0.8
Mean - 2SD	8.3	9.1	8.6
Mean + 2SD	11.5	11.5	11.6

The volume of the 3.1 fractions (after the gelfiltration chromatography) for the different types of IPV is about 10 l. The standard deviation seen for this parameter is small; showing that this process step is under control with regard of this parameter.

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Table 3.5c D-antigen concentration (DE/ml)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	9162	2384	5735
Standard Deviation	2918	373	1567
Mean - 2SD	3326	1638	2600
Mean + 2SD	14997	3130	8869

The amount of D-antigen for type 2 seems to show the highest reproducibility (although the amount of measurement is lowest for this type). For the other types the variability is high.

Table 3.5d E280 (AU) after gel filtration

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	0.988	1.256	1.009
Standard Deviation	0.152	0.184	0.262
Mean - 2SD	0.683	0.889	0.485
Mean + 2SD	1.292	1.624	1.533

The E280 (Extinction at 280 nm) is an indication for the total amount of protein present; this consists of D-antigen and other cellular and viral proteins. It is very likely that the contribution of D-antigen to the E280 is limited in comparison to the other proteins. This can be concluded from the fact that the E280 is about the same for all types of IPV (about 1) and slightly higher for IPV type 2. The D-antigen concentration of the different types shows a big difference, with the D-antigen concentration of type 2 as lowest (Opposite to the results of E 280).
The standard deviation seen for this parameter is completely due to the variation in process, since the technique of measurement (UV-spectrometry) is not likely to show a high variability.
It can be concluded that the standard deviation is relatively high (15-20%), showing that there is variability in the amount of protein present in this step. This is very likely caused by variability in protein concentration (due to variability in cell concentration). Therefore, this variability is acceptable.

Table 3.5e E260 (AU) after gel filtration

Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	1.208	1.425	1.245
Standard Deviation	0.178	0.210	0.255
Mean - 2SD	0.852	1.005	0.736
Mean + 2SD	1.564	1.845	1.755

The E260 (Extinction at 260 nm) is an indication for the total amount of RNA present. The values are about the same for the different types of IPV; with a slightly higher value for the type 2 D-antigen.
The standard deviation seen for this parameter is completely due to the variation in the process, since the technique of measurement (UV-spectrometry) is not likely to show a high variability.
The standard deviations found are about the same as were seen for the E280, this might confirm that the variability of both the E280 and E260 is most likely caused by the variability in starting protein and DNA and RNA concentration (due to variability in cell concentration and virus concentration). Therefore, this variability is acceptable.

Table 3.5f E260/E280 after gel filtration

Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	1.227	1.135	1.259
Standard Deviation	0.077	0.032	0.116
Mean - 2SD	1.072	1.072	1.026
Mean + 2SD	1.381	1.198	1.492

The ratio between E260 and E280 is an indication of the amount of DNA and RNA with respect to the amount of protein. This ratio is about the same for all types of IPV and the variability of this parameter is very low. This shows that there is indeed a correlation between the DNA and the protein concentration at this step and that the purification by the size exclusion chromatography is reproducible.

Conclusion:
The gel filtration step is under control, since all parameters show the same pattern for the different types of IPV and the standard deviations of the results obtained are acceptable.
The variability seen for the E280 and E260 are most likely caused by the variation of cells and virus present. This can be concluded from the fact that the same variability is seen for both parameters and that the ratio (E260/E280) shows a very low standard deviation.

3.6 Ion exchange chromatography

Ion exchange chromatography is performed using Sepharose DEAE-FastFlow as an anion exchange resin. Almost all negative -ions -proteins and -cell components are absorbed by the resin.

Table 3.6a Initial Volume (l)

Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	9.9	10.1	10.1
Standard Deviation	0.7	0.9	0.7
Mean - 2SD	8.5	8.3	8.7
Mean + 2SD	11.3	11.9	11.5

The initial volume applied on the column is the volume as was obtained from the gel filtration step minus the samples taken is about 10 l. It is not expected that small changes in initial volumes on the column will result in a different chromatographic pattern.

Table 3.6b Volume fraction 4.1 (l)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	14.5	14.1	15.2
Standard Deviation	1.3	1.1	2.3
Mean - 2SD	11.9	11.9	10.6
Mean + 2SD	17.1	16.3	19.8

The volume of the 4.1 fractions (after the ion-chromatography step) for the different types of IPV is about 14-15 l. The standard deviation seen for this parameter is small which is expected since the volume collected is controlled with a set point of about 14 to 15 liters.

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Table 3.6c D-antigen concentration fraction 4.1 (DE/ml)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	5015	1713	3130
Standard Deviation	1252	203	625
Mean - 2SD	2510	1306	1881
Mean + 2SD	7519	2120	4379

There is a clear difference in D-antigen concentration between the different types. This is expected, since different types of poliomyelitis virus are used and different types of IPV are produced and measured.

Table 3.6d E280 (AU)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	0.281	0.372	0.244
Standard Deviation	0.037	0.035	0.056
Mean - 2SD	0.207	0.302	0.132
Mean + 2SD	0.356	0.441	0.357

The E280 (Extinction at 280 nm) is an indication of the total amount of protein present; it consists of D-antigen and other cellular proteins.
The standard deviation seen for this parameter is about the same as was seen after the gel filtration step (10-22%). Since the starting material shows about the same variation, this variation is seen as acceptable.

Table 3.6e E260 (AU)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	0.466	0.612	0.407
Standard Deviation	0.065	0.073	0.081
Mean - 2SD	0.337	0.466	0.244
Mean + 2SD	0.596	0.757	0.569

Requirement:
Type 1: E260 x 5000 ≥ 2000 DU/ml (E260 ≥ 0.400)
Type 2: E260 x 2800 ≥ 1000 DU/ml (E260 ≥ 0.357)
Type 3: E260 x 7700 ≥ 1500 DU/ml (E260 ≥ 0.195)

The E260 (Extinction at 260 nm) is an indication of the total amount of RNA present and therefore an indication for the amount of virus present. The values found are about the same for the different types of IPV; with a slightly higher value for the type 2 D-antigen. For type 2 and 3 the mean – 2SD and mean + 2SD are within the requirements. For type 1 the mean – 2SD is outside the set requirements.
The standard deviation seen for this parameter is about the same as was seen after the gel filtration step (12-20%). Since the starting material (retrieved after the size exclusion chromatography) shows about the same variation, this variation is seen as acceptable.

Table 3.6f E260/E280

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	1.658	1.645	1.677
Standard Deviation	0.051	0.089	0.086
Mean - 2SD	*1.556*	*1.467*	*1.506*
Mean + 2SD	1.759	*1.823*	*1.849*

Requirement: ≥ 1.60 and ≤ 1.80

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The ratio between E260 and E280 is an indication of the amount of RNA with respect to the amount of proteins. This ratio is about the same for all types of IPV. The ratio increased with about 133-145% compared to the ratio before the ion-chromatography step (see table 3.5f), showing that virus is being purified from proteins and cell components. This shows that this chromatography step is suitable for polio virus purification.
Furthermore, the variability of this parameter is very low and is acceptable for this parameter.

With one exception all means ± 2SD for type 1, type 2 and type 3 polio are outside the limits of the requirements/specifications set for this value, indicating that the control of the process is not adequate compared to the specifications set. Two of the batches produced had an E260/E280 ratio outside the requirements therefore an investigation should be started.

Conclusion:
The purpose of the ion chromatography step is to purify the polio virus from proteins and cell components. The results of the E260, E280 and the E260/E280 show that indeed a purification of protein is obtained. All parameters of the ion exchange chromatography show the same pattern for the different types of IPV and the standard deviations of the results obtained are acceptable
However statistically the means ± 2SD of E260/E280 are for most of the polio types outside the range of the specifications. Therefore an investigation should be started.

3.7 Sterile Filtration

The purified virus is diluted with M199-buffer to a preset concentration. The preset value is type specific: Type 1: 2000 DE/ml, Type 2: 1000 DE/ml and type 3: 1500 DE/ml. To define the desired end volume the current D-antigen concentration is calculated by multiplication of the E260 value with a type specific F factor = dilution factor). On the diluted virus a sterile filtration through a 0.22 µm filter is performed.

Table 3.7a Filtration time (min)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	39	35	37
Standard Deviation	6	6	9
Mean - 2SD	27	23	19
Mean + 2SD	51	47	55

The mean filtration time is between 35-40 min for all types of IPV. The filtration time depends on the air pressure which is used to press the purified virus through the filter and the amount of material.

Table 3.7b Volume 5.1 fraction (l)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	28	24	33
Standard Deviation	5	1	6
Mean - 2SD	18	22	21
Mean + 2SD	38	26	45

The final volume of the 5.1 fraction depends on the F factor, the volume of the purified virus and the E260 value. The relative standard deviation of type 1 and 3 is about 17 % and the standard deviation of type 2 is about 4 %.Since the relative variation of the E260 value is about 12 – 20% the variation in this parameter is acceptable.

Table 3.7c pH

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	6.9	6.9	6.9
Standard Deviation	0.0	0.0	0.0
Mean - 2SD	6.9	6.9	69
Mean + 2SD	6.9	6.9	6.9

The pH after filtration can be set by the addition of sodium bicarbonate. During production of the evaluated batches addition of sodium bicarbonate for pH adjustment has not been necessary. The pH shows a very low relative standard deviation (< 1%) which shows that this parameter is under control.

Table 3.7d D-antigen concentration fraction 5.1 (DE/ml)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	3100	981	1487
Standard Deviation	487	187	341
Mean - 2SD	2126	606	805
Mean + 2SD	4074	1355	2169

There is a clear difference in D-antigen concentration between the different types. This is expected, since different types of poliomyelitis virus are used and different types of IPV are produced and measured.
The relative standard deviation is as expected (about 20%) with regard to the parameter measured and the method used for analyzing D-antigen

Table 3.7e Virus titration (10logCCID50/ml)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	10.06	10.25	9.80
Standard Deviation	0.25	0.19	0.19
Mean - 2SD	9.56	9.86	9.43
Mean + 2SD	10.56	10.63	10.18

Requirement: ≥7 and ≤ 11 10logCCID$_{50}$/ml

The amount of living virus/ml in the different types of IPV is about the same and all results found are well within the specifications. The standard deviation found for this parameter is very low, showing that the complete process of titration, virus growth is under control.
The mean± 2SD is within the set specifications for all types of IPV.

Conclusion:
The sterile filtration step is under control.

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3.8 Inactivation

Inactivation with formaldehyde at 37 °C takes place for 13 days (= approximately 312 hours) with in between a sterile filtration after 6 to 9 days through a 0.22 µm filter. As part of the release testing at 10 days (3 days before end of inactivation) and at 13 days (end of inactivation) the compendial inactivation test is performed on vero cells to proof that no live virus is present. During inactivation a quantitative inactivation test on vero cells is used to determine the inactivation curve.

Table 3.8.a Inactivation curve type 1 (10logCCID50/ml; n = 6)

	t = 0	t = 24	t = 48	t = 72	T = 98	t = 120
Mean	9.42	6.71	4.46	2.08	n.d.	n.d.
Standard Deviation	0.40	0.32	0.28	0.64	n.a	n.a.
Mean - 2SD	8.62	6.07	3.90	0.79	n.a	n.a.
Mean + 2SD	10.21	7.34	5.02	3.37	n.a	n.a.

Table 3.8.b Inactivation curve type 2 (10logCCID50/ml; n = 4)

	t = 0	t = 24	t = 48	t = 72	T = 98	t = 120
Mean	10.02	7.17	4.93	2.34	n.d.	n.d.
Standard Deviation	0.23	0.01	0.18	0.52	n.a	n.a.
Mean - 2SD	9.57	7.14	4.57	1.30	n.a	n.a.
Mean + 2SD	10.47	7.20	5.28	3.37	n.a	n.a.

Table 3.8.c Inactivation curve type 3 (10logCCID50/ml; n = 10)

	t = 0	t = 24	t = 48	t = 72	T = 98	t = 120
Mean	9.53	6.68	4.30	1.44	n.d.	n.d.
Standard Deviation	0.27	0.36	0.31	0.44	n.a	n.a.
Mean - 2SD	8.99	5.96	3.68	0.56	n.a	n.a.
Mean + 2SD	10.07	7.39	4.93	2.32	n.a	n.a.

n.d. = non detected

The inactivation curves shows a quick reduction of the live virus concentration. Within 120 hours the concentration of live virus is under the detection limit of this quantitative test. Testing with this test on time points later than t=120 does not add any information. The amount of live virus is under the detection limit of the in-process test after 120 hours and the inactivation is performed for 13 days (= approximately 312 hours). This is an enormous over kill. After 10 days (approximately 240 hours) no live virus was present. See also table 3.9.4a.
Therefore, it can be concluded that all types of IPV are always completely inactivated.

3.9 Release tests and critical IPC tests

Only batches that were not aborted during production are taken into account with respect to the evaluation of the results of the release tests. During production 5 batches were aborted: 1 due to microbiological contamination during cell culture, 3 due to contamination during down stream processing and one due to a deviation caused by documentation issues.

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3.9.1 Requirement on Vero cells:

Table 3.9.1a Overview of the results of the release tests on Vero cells of not aborted batches

Test	Requirement	Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Extraneous viruses (ANA-22453)	No extraneous viruses detected	% pass	100	100	100
Mycoplasma (ANA-10159)	No growth of mycoplasma	% pass	100	100	100
Identity (ANA-10067)	Vero cells	% pass	100	100	100

The control cells were tested for extraneous viruses, identity and mycoplasma. In all batches produced, no extraneous viruses and no mycoplasma were detected.
Furthermore, the cells were identified as Vero cells.

Conclusion:
From these results it can be concluded that the Vero cells cultivated are fulfilling the requirements.

3.9.2 Requirements on Virus harvest

Table 3.9.2a Overview of the results of the release test on Virus harvest of not aborted batches

Test	Requirement	Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Extraneous viruses (ANA-10295)	No extraneous viruses detected	% pass	100	100	100
Sterility (ANA-10158)	No growth of bacteria or fungi	% pass	100	100	100

The virus harvests were tested for extraneous viruses and sterility. In all batches produced, no extraneous viruses were present.
In 1 out of the 25 batches the batch was aborted due to microbial contamination of the culture. This contamination was observed in the culture phase. The virus harvest of all other batches showed to be sterile.

Conclusion:
From these results it can be concluded that the virus harvest is fulfilling the requirements.

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3.9.3 Requirements on Virus harvest, concentrated and purified

Table 3.9.3a Overview of the results of sterility and identity on concentrated and purified virus of not aborted batches

Test	Requirement	Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Sterility (ANA-10158)	No growth of bacteria or fungi	% pass	100	100	100
Identity (ANA-10096)	Specific IPV type	% pass	100	100	100

The concentrated and purified virus harvests were tested for sterility; all batches tested did not show any growth of bacteria or fungi.
3 out of the 25 batches were aborted during concentration and purification due to microbial contamination of the culture. In all three cases, this contamination was due to different technical issues that have been resolved afterwards. In all other cases the batches produced resulted into sterile monovalent bulk.

D-antigen 4.1 fraction according to ANA-20002
The results of this test have been discussed under table 3.6c.

Table 3.9.3b Protein Nitrogen content (mg/l)

parameter	Type 1 (n=3)	Type 2 (n=2)	Type 3 (n=8)
Mean	8,40	10,13	5,96
Standard Deviation	0,20	1,23	1,62
Mean - 2SD	8,00	7,67	2,72
Mean + 2SD	8,81	12,59	9,20

No requirements

Table 3.9.3c Specific activity (DE/mgPN)

parameter	Type 1 (n=3)	Type 2 (n=2)	Type 3 (n=7)
Mean	565000	166000	367000
Standard Deviation	138000	37600	78100
Mean - 2SD	290000	90400	210000
Mean + 2SD	840000	241000	523000

Requirements
Type 1: > 300000 DE/mgPN
Type 2: > 100000 DE/mgPN
Type 3: > 250000 DE/mgPN

The Protein Nitrogen content and the D-antigen content have been measured. The specific activity has been calculated. The results are shown in table 3.6c, 3.9.3b and 3.9.3c.
At the moment of this report only a part of all batches produced have been tested therefore n is relatively small resulting in higher RSD's. An evaluation of the individual results shows that for all types all results are well above the requirements. Therefore it can be concluded that this parameter is under control.

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E260 value (critical IPC)
The results of this test have been discussed under table 3.6e

Ratio E260/E280 (critical IPC)
The results of this test have been discussed under table 3.6f

Test on Virus titration 5.1 fraction according to ANA-10101 (in [10logCCID50/ml)
The results of this test have been discussed under table 3.7e.

Conclusion:
From these results it can be concluded that the concentrated and purified harvest is fulfilling the requirements for all tests except for 2 batches that do not fulfill the requirements for E260/E280.
In addition, suitable measures have already been taken to resolve the technical issues related to the high number (4 out of 25) of contaminated batches. Since May 2007 no contamination was found.

3.9.4 Requirements on Monovalent pool

Table 3.9.4a Overview of the results on sterility, Inactivation and DNA impurity of the monovalent bulk

Test	Requirement	Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Sterility (ANA-10158)	No growth of bacteria or fungi	% pass	100	100	100
Inactivation (5.1 fraction, 3 days before end of inactivation; ANA-10097)	No CPE due to poliovirus detected	% pass	100	100	100
Inactivation (6.1 fraction; ANA-10097)	No CPE due to poliovirus detected	% pass	100	100	100
DNA impurity level (ANA-21606)	≤ 100 pg/human dosage (calculated)	% pass	100	100	100

The monovalent pools were tested for sterility. In all batches produced, no growth of micro organisms was seen.
All batches produced fulfill the specification regarding DNA impurity present.
All batches produced fulfill the specifications regarding inactivation (see also § 3.8)

Table 3.9.4b: Test on formaldehyde according to ANA-21745 (in mmol/l)

Parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	2.9	2.8	2.8
Standard Deviation	0.1	0.1	0.3
Mean - 2SD	2.7	2.6	2.2
Mean + 2SD	3.1	2.9	3.2

Requirement: > 2 mmol/l

The formaldehyde concentration found in the monovalent pools is well within the specifications. The standard deviation found for type 1 or type 2 is small. For type 3 a larger standard deviation is found. Looking in the individual results, it seems that this larger standard deviation is mainly caused by one aberrant result (2.2 mmol/l).
From the results obtained, it can be concluded that the process is under control regarding this parameter.

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Table 3.9.4c: Release test on D-antigen according to ANA-10102 (in DE/ml)

parameter	Type 1 (n=6)	Type 2 (n=4)	Type 3 (n=10)
Mean	2747	1032	1488
Standard Deviation	271	87	147
Mean - 2SD	2205	858	1193
Mean + 2SD	3289	1206	1782

Requirement:
Type 1: 1250 – 3140 DE/ml
Type 2: 430 – 1480 DE/ml
Type 3: 520 – 2220 DE/ml

The standard deviation found for the D-antigen for the different IPV-types is relatively small (<10%) with regard to the method used. Furthermore, for the type 2 and type 3 the mean – 2SD and the mean + 2SD are well within the preset specifications. Therefore, it can be concluded that the process for this parameter is under control.
However, the Mean + 2SD of the type 1 D-antigen is above the pre-set specification. This might become a problem for future batches. Although only 6 batches are used for this calculation, it is not expected that a higher number of batches will result in a lower standard deviation, since a 10% standard deviation for D-antigen measured with an ELISA test is an acceptable value.
One batch was already rejected because of the fact that the D-antigen level was too high. It is recommended to investigate this issue.

4 Conclusion

The production process of IPV monovalent bulk production on Vero cells on a 1500 liter scale is considered under control. Based on the results several recommendations can be made to improve the efficacy. See chapter 5.

5 Recommendations

Ion exchange chromatography

- An investigation should start whether the specifications set for E260/E280 are too strict or whether it is possible to further optimize the process.

Release tests

- It is recommended to investigate the issue regarding the high D-antigen content in the monovalent bulk for IPV type 1 in relation to the predefined specifications.

6 Archive Raw Data

Not applicable

7 Attachements

Not Applicable

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3.2.S.2.5. Validación o evaluación del proceso

2 **Consistencia en la fabricación de mezclas monovalentes de la VPI con células Vero a una escala de 700 litros**

La validez del proceso de producción con células Vero se demuestra por la consistencia en los resultados de 6 lotes de mezclas monovalentes a una escala piloto de 150 litros (dos lotes tipo 1, dos lotes tipo 2, dos lotes tipo 3; tabla 1) y 17 lotes de mezclas monovalentes a una escala de producción de 700 litros (seis lotes tipo 1, en la tabla 2; cinco lotes tipo 2, en la tabla 3; y seis lotes tipo 3, en la tabla 4).

Los procesos analíticos mencionados en las tablas a continuación se describen en el Módulo 3.2.S.2.4 (Control de pasos críticos y productos intermedios).

La consistencia en los lotes de fabricación de 700 y 1500 litros cultivados con células de riñón de mono (CRM) se incluye en los apéndices 1 y 2.

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