3.2.A Appendices

3.2.A.2 Adventitious Agents Safety Evaluation

The safety of the KN035 has been ensured through its DS and DP manufacturing process and raw materials controls. The manufacturing of KN035 is conducted in a strict cGMP environment to minimize the introduction of adventitious agents during the manufacturing process.

The raw materials are carefully screened and tested especially for the animal sourced materials. Animal sourced materials (two lots of FBS and one lot of Trypsin) were used in the host cell line CHOK1 adaptation and growth. One lot of FBS (Hyclone) is sourced from South America where no BSE is known to exist. This lot of FBS (lot NUK0201) is tested to be negative for sterility, endotoxin, mycoplasma, and bovine viral diarrhea virus. The other lot of FBS (lot DPH0176) from Hyclone is sourced from New Zealand, which is a BSE free country, and was tested to be negative for endotoxin, sterility, mycoplasma and bovine virus as per 9 CFR 113. The CoAs from both lot of FBS are attached in section 3.2.A.4.1.The trypsin (lot 774748) used during the host cell line adaptation is tested to be negative by in vitro bioassay, mycoplasma, sterility and porcine parvovirus (3.2.A.4.2).

The WCB of the host cell line CHOK1S was tested of free of Bornavirus, porcine virus and bovine/porcine circovirus (PCV) (Table 3. 15)

No animal sourced materials were used after host CHO cell line establishment (CHOK1S) process. In addition, the MCB of KN035 is extensively tested for bovine virus again as per 9 CFR 113and bovine polyoma virus.

No animal sourced materials were used in the manufacturing process of KN035.

The unprocessed bulk after production bioreactor is tested to be free from adventitious virus by in vitro and in vivo tests. The results are presented in Table 3.75 below.

All the test report of MCB, WCB and unprocessed bulk are attached in section 3.2.A.5.1, 3.2.A.5.2 and 3.2.A.5.3

The manufacturing process has at least 3 viral reduction steps: low pH inactivation, ion exchange chromatography and nanofiltration with the pore size of 20 nm. The viral clearance study results and qualification of scale down models are presented in the following sections. The reports are attached in section 3.2.A.5.4

Unprocessed Bulk

Unprocessed bulk samples were collected prior to any centrifuge or clarification. The unprocessed bulk testing was performed by BioReliance (Table 3.75).

Table 3. 75 Unprocessed bulk testing of lot 151202DS

Test Item	Method	Acceptance Criteria	Results
Bioburden	USP <61>	< 10 CFU/mL	0 CFU/mL
Mycoplasma (cultivable and non-cultivable)	USP and PTC 1993[1]	Not detected	Not detected
In vitro assay for adventitious virus	In vitro assay for viral contaminants using MRC-5, VERO, CHO cells (28 days)	Not detected	Not detected
MMV virus detection	qPCR	Negative	Negative
Retrovirus virus particle detection	Thin section transmission electron microscopy (TEM)	Report results	5.6 X 10^6 retrovirus-like particles (VLPs)/mL
In vivo adventitious virus test	Sucking mice, adult mice, guinea pigs and embryonated eggs	Not detected	Not detected

1. FDA points to consider in the characterization of cell lines used to produce biologicals (1993)

Viral Clearance Study

Viral clearance studies were performed for the low pH inactivation step, anion exchange chromatography and the nanofiltration step in small scale validation studies by BioReliance. The materials used in the viral clearance study are derived from the manufacturing intermediate from clinical DS lot 151202DS. The critical parameters used in the scale-down model are presented in Table 3.76 to Table 3.78.

Table 3. 76 Process parameters used in the small-scale viral validation studies for low pH inactivation

Process Parameters	250 L Manufacturing Process	Scale Down Process
pH	3.6 -3.7	3.7
Inactivation time	60 -90 min	60 min
Temperature	18°C -25 °C	18-25°C

Table 3. 77 Process parameters used in the GigaCap Q-650 AEX small-scale viral validation studies

Parameter	Buffer	250 L Pilot Scale	Scale Down Process
Column Bed height specification Column dimension (d x h cm) Column volume (CV) Scale down factor	N/A	 25 ± 5 cm 30 x26 20.9 L N/A	 25 ± 5 cm 1.1 x 20 19.0 mL 1:1100
Linear flow rate (excluding 0.5 M NaOH)	N/A	300 cm/h	300 cm/h

Parameter	Buffer	250 L Pilot Scale	Scale Down Process
Equilibration buffer **(5CV)** Effluent pH Target effluent conductivity	10 mM Tris HCl, pH 7.5	80 L pH 7.40 to 7.60 1.486 mS/cm	95 mL pH 7.40 to 7.60 1.486 mS/cm
Loading KN035 mg/mL	N/A	≤40 g/L	40.0 g/L
Peak collection criteria	N/A	0.1AU↑ 0.1AU↓	0.1AU↑ 0.1AU↓

Table 3. 78 Process parameters used in the scale down models of viral reduction nanofiltration

Parameter	Buffer	250 L Pilot Scale	Scale Down Process[1]
Filter area	N/A	0.2m^2	5cm^2
Water flush	WFI	50L/m^2	≥50 mL
Water flush pressure	N/A	2.0bar, 29psi	2.0bar, 29psi
NWP(Normalized Water Permeability)pressure	N/A	2.0bar, 29psi	1.0bar, 14.5psi
Filter equilibration	10 mM Tris, pH 7.5	100L/m^2	50mL
Equilibration pressure	N/A	2.0bar, 29psi	2.0bar, 29psi
Filter load	KN035BNL	18.2mg/mL 255L/m^2	19.6mg/mL 660L/m^2 (330 mL)
KN035 BNL[2] filtration pressure	N/A	2.0bar, 29psi	2.0bar, 29psi
Filter rinse	10 mM Tris, pH 7.5	20L/m^2	20L/m^2 (10mL)
Rinse filtration pressure	N/A	2.0bar, 29psi	2.0bar, 29psi
Scale down factor	N/A	N/A	1:400

1. For the scale down filtration, the minimum specified pressure will be used in the study as the "worst case"
2. BNL: batch nanofiltration load. It means the product intermediate prior to the nanofiltration

Filter load of 660 L/m^2 and normalized water permeability (NWP) of 1 bar and 14.5 psi were used in the small scale validation study to represent the worst case scenario.

Xenotropic Murine leukemia virus (XMuLV) and Murine minute virus (MMV) were used in the small scale viral clearance studies (Table 3.79).

Table 3. 79 Virus used in the viral clearance studies

Viral Names	Genome	Envelope	Family	Size(nm)	Chemical Resistance
XMuLV	ssRNA	Yes	Retroviridae	80-110	low
MMV	ssDNA	No	Parvoviridae	18-24 nm	Very high

Prior to the viral clearance studies, the samples are tested for viral interference and cytotoxicity to the indicator cell lines used in the study. Appropriate dilution was used when the potential cytotoxicity to the indicator cells is suspected. The viral clearance study results are summarized in Table 3.80. The complete study report prepared by BioReliance can be provided upon request.

Table 3. 80 Log reduction factor summary

TABLE 1A – LOG REDUCTION FACTORS		
LOW pH TREATMENT	**RUN 1**	**RUN 2**
MLV	4.51 ± 0.39 $\log_{10}$	4.89 ± 0.44 $\log_{10}$
ANION EXCHANGE CHROMATOGRAPHY		
MLV	≥5.42 ± 0.33 $\log_{10}$	≥5.41 ± 0.31 $\log_{10}$
MMV	5.29 ± 0.36 $\log_{10}$	6.07 ± 0.36 $\log_{10}$
VIRUS REDUCTION FILTRATION		
MLV	≥3.79 ± 0.33 $\log_{10}$	≥3.53 ± 0.32 $\log_{10}$
MMV	≥6.84 ± 0.29 $\log_{10}$	6.25 ± 0.44 $\log_{10}$

TABLE 1B – OVERALL LOG REDUCTION FACTORS	
MLV	≥13.45 ± 0.59 $\log_{10}$
MMV	11.54 ± 0.57 $\log_{10}$

Safety margin calculation

Maximum load of virus like particles in the unprocessed bulk of clinical DS batch 151202DS determined by TEM is: 5.6 X 10^6 particles/mL(A)
Maximum single dose proposed is 10 mg/kg, for a 70 kg human subject, it is 700 mg.
Yield from manufacturing process %:74.5%
Concentration of KN035 at the time of harvest: 6.56 g/L
Total volume of unprocessed bulk needed to make one maximum single dose: 700 mg/ (6.56 mg/mL X0.745) = 143.2 mL (B)
Log reduction factor = 13.45 (C)
Viral particles pre single maximum does: = A XB /C=

$$\frac{5.6 \times 10^6 \text{ viral particles/mL} \times 143.2 \text{ mL}}{10^{13.45}}$$

= 2.85 X 10^{-5} viral particles per dose

[KN035, Alphamab Co., Ltd]

Which estimates to be 1 viral particle per 35145 doses.