

As shown in the Table below (Table 1), the manufacturing procedure for Berinert P was divided into different stages. Two steps in the manufacturing process were investigated for their capacity to clear viruses, Pasteurization and Hydrophilic interaction

PRV, which is inactivated within -b(4)- with mean inactivation factor of 6.3 log₁₀ has to be considered as a peculiarity due to the fact that in the presence of --b(4)-- PRV is forming (heat stable) aggregates.

As demonstrated in experiments utilizing ---b(4)----- material, PRV is very heat sensitive; therefore, residual virus infectivity after the pasteurization step of Berinert P is unique for this non-specific model virus. *Nevertheless, PRV is very effectively inactivated by pasteurization under the specific conditions for Berinert P.*

The non-enveloped virus HAV is very effectively inactivated by pasteurization whereas the non-enveloped parvovirus CPV was inactivated by 1.4 log₁₀.

It has been shown that pasteurization inactivates $\geq 7.0 \log_{10}$ of WNV infectivity.

Partitioning (Hydrophobic interaction chromatography (HIC)):

For all enveloped viruses, no residual infectivity could be demonstrated in the C1-INH containing elute, demonstrating a mean virus reduction factor of $\geq 4.5 \log_{10}$ for HIV, of $\geq 4.6 \log_{10}$ for BVDV and $\geq 6.5 \log_{10}$ for PRV. Virus infectivity, however, was detected in this final sample for the non-enveloped viruses HAV and CPV. Nevertheless, these non-enveloped viruses were removed by this chromatography resulting in a mean virus reduction factor of 4.5 log₁₀ for HAV and 6.1 log₁₀ for CPV. All spiked virus could be recovered in the discarded side fraction from column regeneration; sanitization of the column ---b(4)----- resulted in the inactivation of all viruses spiked onto the column, demonstrating an effective sanitization of the column by the regeneration /sanitization cycles as performed in production.

Table 2: Validated steps and their viral clearance capacity for a given virus

Virus	Pasteurization *		HIC	Total Log Reduction
	Log Reduction	Time to inactivation (hour)		
HIV	³ 6.6	b(4)	³ 4.5	³ 11.0
BVDV	³ 9.2	b(4)	³ 4.6	³ 13.8
PRV	³ 6.7	b(4)	³ 6.5	³ 13.2
HAV	³ 6.4	b(4)	4.5	³ 10.9
CPV	1.4	b(4)	³ 6.1	7.5
WNV	³ 7.0	b(4)	Not done	N/A

* The --b(4)--- concentration is -----b(4)----- product solution; this is defined as ----b(4)----- (representing an e.g. final ----b(4)--- concentration of appr. --b(4)--

Validation of HIV reduction: Module 3 Volume 17; Virus Validation Report VVR-617-HIV-02:

Pasteurization Conditions for HIV clearance experiments

[b(4)]

Note: in some experiments, residual virus remained after -b(4)- of heat treatment under production conditions. Similar results have been obtained in the robustness conditions -----b(4)----- In addition, as shown in the figure below, the heat treatment has been slow in inactivating HIV, and that up to -b(4)----- of heat is needed to inactivate HIV, under production conditions. Historically, one expects HIV inactivation within the

first hour of heat treatment. It appears that high level of stabilizers have greatly slowed the inactivation process, likely by --b(4)---- the virus.

1 Page determined to be not releasable: b(4)

The --b(4)-- concentration is determined by adding exactly ---b(4)-----
----- product solution; this is defined as --b(4)----- concentration
(representing an e.g. final ---b(4)---- concentration of appr. ---b(4)-----
PRV can be stabilized to a very high degree against heat inactivation by --b(4)----- like
--b(4)--. To find out which --b(4)-- is responsible for the PRV ---b(4)----- the
following experiments were done: in this series the ---b(4)----- was studied
separately by spiking various concentrations of solely aqueous --b(4)-- solutions (--b(4)-
-----) with PRP and then subjected to pasteurization. The results shown in Figure 12
indicate that ----b(4)----- PRV against heat inactivation.

As shown above HIV was inactivated after --b(4)--, which indicate that the virus is more
heat stable in Berinert P --b(4)----- than has been reported for other products (including
all other CSL products).According to the sponsor, the stabilizing conditions for HIV are
unknown. The sponsor speculated that the C1-INH molecule itself may have HIV –
stabilizing impact, indicating that C1-INH binds directly to HIV (data not provided). As
shown in the figure below, the stabilizing conditions appear to be different depending on
the production lot. This further underscores the lack of robustness in the inactivation
process.

1 Page determined to be not releasable: b(4)

PRV is stabilized to a very high degree against heat inactivation --b(4)----. The sponsor
has performed experiments in which series the --b(4)----- was studied separately
by spiking various concentrations of solely aqueous --b(4)-- solutions (--b(4)-----)
with PRP and then subjected to pasteurization. The results shown in Figure 12 indicate
that --b(4)----- PRV against heat inactivation. Under production conditions, PRV
inactivation achieved after --b(4)--, with some residual virus remaining.

[b(4)]

1 Page determined to be not releasable: b(4)

[b(4)]

Comments & Proposed Recommendations after 1st Cycle Review:

The combined affect of Pasteurization and HIC (chromatography) has resulted in >11
log reduction for HIV, BVDV and PRV the three envelope viruses, this indicates an
acceptable degree of clearance for these viruses.However, review of the viral validation
data point to unexpected heat stability of HIV and PRV in the pasteurization step under
the current manufacturing conditions. The other validated virus clearance step is
chromatography, which like most removal steps is inherently less reliable.

Therefore, additional inactivation/removal step (or steps) in the manufacturing process
is needed to provide higher assurance of viral safety for this product. We proposed the
inclusion of -----b(4)-----

As indicated in the CR letter, because of unexpected heat stability of HIV and PRV in the pasteurization step and the limitations of HIC, the agency asked CSL to validate additional manufacturing steps for their capacity to clear HIV and PRV. In response the sponsor has validated DEAE-Sephadex A50 Chromatography, QAE-Sephadex Chromatography and Ammonium Sulphate Precipitations steps in the manufacturing process ((Steps 2-4, see Table 1) for their combined capacity to clear HIV and PRV. These manufacturing steps have also been studied separately (as a single step) to determine their individual contribution to clearance of HIV and PRV. Validation data demonstrated that these manufacturing steps contribute to the removal of HIV and more significantly to the removal of PRV. The manufacturer has also made a post approval commitment -----b(4)-----

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Summary of virus validation data for HIV and PRV that are submitted in response to the CR letter is outlined below:

HIV clearance by DEAE-Sephadex A50 Chromatography, QAE-Sephadex Chromatography and Ammonium Sulphate Precipitations (Stages b(4)) Studied in Combination:

The removal of HIV was investigated by the combination of DEAE-sephadex A50 chromatography, QAE-sephadex chromatography and ammonium sulphate precipitations. For this study, --b(4)----- from production lots was spiked with HIV and processed to the final dissolved ammonium sulphate precipitate as defined by the production process. This final sample and the other fractions of the partitioning steps were assayed for HIV. Based on two separate experimental data, HIV clearance of 4.6 logs and 4.0 logs were obtained respectively, with average clearance rate of 4.3 log₁₀ (see Table 3). Protein content of starting material and the final sample were used to establish the relevance of the small scale model to the actual process (downscale validation DSVR -617-001-01).

Validation data for individual steps (DEAE-Sephadex A50 Chromatography, QAE-Sephadex Chromatography and Ammonium Sulphate Precipitations) have demonstrated that QAE, ammonium sulphate precipitation, and DEAE –Sephadex, when studied separately contributed only to a limited extent to HIV clearance. However their combine viral clearance effect is more than 4.0 logs (see Table 3).

PRV clearance by combination of DEAE and QAE Chromatography followed by Ammonium Sulphate Precipitations (Stage -b(4)-):

The removal of PRV was investigated by the combination of DEAE-sephadex A50 chromatography, QAE-sephadex chromatography and ammonium sulphate precipitations. For this study, ---b(4)----- from production lots was spiked with PRV and processed to the final dissolved ammonium sulphate precipitate as defined by the production process. This final sample and the other fractions of the partitioning steps were assayed for PRV. Based on two separate experimental data, PRV clearance ≥ 7.7 log and ≥ 6.6 log were obtained respectively, with average clearance rate of ≥ 7.2 log.

(See Table 3). Protein content of starting material and the final sample were used to establish the relevance of the small scale model to the actual process. Validation data for individual steps (DEAE-Sephadex A50 Chromatography, QAE-Sephadex Chromatography and Ammonium Sulphate Precipitations) have demonstrated that QAE, and DEAE Sephadex, contribution to viral clearance is limited. However, Ammonium Sulphate precipitation contributed significantly to the clearance of PRV. Mean individual reduction factors for these steps shown below:

DEAE chromatograph of --b(4)---,

QAE chromatograph of --b(4)---,

ammonium sulphate precipitations of --b(4)--.

In addition to validation of viral clearance capacity of the manufacturing process for DEAE and QAE Chromatography followed by ammonium sulphate precipitations (stage -b(4)-), CSL has also made a post marketing commitment (PMC) to --b(4)-----

Table below demonstrate total viral clearance in the manufacturing process of C1 Esterase Inhibitor (Human), Berinert® including clearance data submitted for HIV-1 and PRV in response to the CR letter.

Table 3: Mean Virus clearance in the manufacturing process of C1 Esterase Inhibitor (Human), Berinert® (with revised HIV-1 and PRV clearance level)

Virus	Pasteurization	HIC	DEAE-Sephadex A50 chromatography QAE-sephadex chromatography Ammonium Sulphate Precipitation	Total Log Reduction
HIV	³ 6.6	³ 4.5	4.3	³ 15.4
BVDV	³ 9.2	³ 4.6		³ 13.8
PRV	6.3	³ 6.5	³ 7.7	³ 20.5
HAV	³ 6.4	4.5		³ 10.9
CPV	1.4	³ 6.1		7.5
WNV	³ 7.0	Not done		N/A

Final Recommendation:

The approval of the product with regard to viral validation studies is recommended. This recommendation is based on recent and additional viral validation data, obtained for the removal of HIV, and PRV at stages -b(4)- of the manufacturing process. These data

have completed earlier viral validation data generated for the pasteurization and HIC steps in the manufacturing process (see Table 1). The recommendation also is based on CSL's post approval commitment to -----b(4)-----

