Supporting Information (SI) File

Worst Case Conditions for Viral Clearance

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SUPPORTING INFORMATION

What are the worst case conditions looked at during validation and does this differ depending on the type of filing?

Question 3a Please indicate the Worst Case Conditions for Virus Inactivation:

Virus Inactivation

Worst Case Condition Parameters
Notes: please answer with what you include in your health authority submissions
select the appropriate answer from the drop down boxes (Low, High, Not Considered*)
*Not considered means that it is not considered necessary as part of the study to define worst case

Method	Filing Type	pH Low	pH High	pH Not Considered	Agitation Low	Agitation High	Agitation Not Considered	Conductivity Low	Conductivity High	Conductivity Not Considered	Protein Concentration Low	Protein Concentration High	Protein concentration Not Considered
pH	IND	1	13	1	0	0	15	0	0	15	0	2	13
	BLA	1	14	0	0	1	14	0	0	15	0	3	12
Detergent/ Solvent	IND	2	0	9	2	0	9	0	0	11	0	2	9
	BLA	2	0	10	2	0	10	0	0	12	0	4	8
UV	IND	0	0	2	0	0	2	0	0	2	0	0	2
	BLA	0	0	2	0	0	2	0	0	2	0	0	2

Method	Filing Type	Inactivation Time Low	Inactivation Time High	Inactivation time Not Considered	Temp Low	Temp High	Temp Not Considered	Intensity Low	Intensity High	Intensity Not Considered	Flow Rate Low	Flow Rate High	Flow Rate Not Considered
pH	IND	15	0	0	10	1	4						
	BLA	15	0	0	13	1	1						
Detergent/ Solvent	IND	11	0	0	9	0	2						
	BLA	12	0	0	10	0	2						
UV	IND	0	0	2	0	0	2	0	0	2	0	1	1
	BLA	0	0	2	0	0	2	0	0	2	0	1	1

Method	Filing Type	Volume added (based on % or ratio) Low	Volume added (based on % or ratio) High	Volume added (based on % or ratio) Not Considered
pH	IND			
	BLA			
Detergent/ Solvent	IND	8	0	1
	BLA	9	0	1
UV	IND			
	BLA			

Please add any additional comments regarding Virus Inactivation including reasons and justification for approach	file inactivation kinetics * * * wrt the "volume added" for detergent, virus validation study is performed at a lower detergent concentration than our mfg target. * * * * * * Usually no mixing during inactivation, so no mixing at small scale inactivation. Mixing to achieve homogeniety is a separate from the inactivation evaluation. * * We have neveer performed UV method. * * * * * * * Temperature is controlled within the range of 15-20 C during spiking studies, and we argue in filings that this represents full scale mfg process. * * * For UV inactivation: Lamp intensity is a property of equipment. It is fixed. UV dose is a function of Residence time (Flow rate) and product properties (OD) => More generally, make Flow rate vary is sufficient to test lowest dose. * * Agitation only performed during pH adjustment for 'sufficient' mixing. Incubation with no agitation. Ambient temp typically used for early phase testing. *

		Filing Type	ASTM Used (for pH viral inactivation)? Yes	ASTM Used (for pH viral inactivation)? No	Details/ Country Restrictions
Question3b	For pH Viral Inactivation: Are you filing Virus Clearance using the ASTM standard practice?	IND	2	13	FDA * * * * We plan to in the future. * * * * practices are aligned with ASTM * Submissions are in countries. * In future will consider ASTM * * * * * * We aim for global submission and don't want to take the risk by using ASTM alone. * * * * * * * To date: Project/molecule dependent conditions (mainly pH) chosen and tested. Though where possible the ASTM standard practice is targeted. *
		BLA	0	14	* * * * * * * * practices are aligned with ASTM * Submissions are in countries. * In future will consider ASTM * * * * * * We aim for global submission and don't want to take the risk by using ASTM alone. * * * * * * * *

		Filing Type	ASTM Used (for Triton X-100)? Yes	ASTM Used (for Triton X-100)? No	Details/ Country Restrictions
	For Triton X-100 viral inactivation: Are you filing Virus Clearance using the ASTM standard practice?	IND	0	8	* * * We have moved away from TX100 as a detergent for inactivation. * * * * * * na * * * * * * * We aim for global submission and don't want to take the risk by using ASTM alone. * * * * * * * *
		BLA	0	9	* * * * We are moving away from use of Triton X-100 in our platform process. * * * * * na * * * * * * * We aim for global submission and don't want to take the risk by using ASTM alone. * * * * * * * *

Question 3c

Please indicate the Worst Case Conditions for Bind and Elute Chromatography

Bind and Elute Chromatography		Worst Case Condition Parameters Notes: please answer with what you include in your health authority submissions select the appropriate answer from the drop down boxes (Low, High, Not Considered*) *Not considered means that it is not considered necessary as part of the study to define worst case											
Method	Filing Type	Resin Load Density Low	Resin Load Density High	Resin Load Density Not Considered	Resin Load Volume Low	Resin Load Volume High	Resin Load Volume Not Considered	Protein load concentration Low	Protein load concentration High	Protein load concentration Not Considered	Load pH Low	Load pH High	Load pH Not Considered
Affinity (Protein A)	IND	2	8	4	1	2	11	1	1	12	0	0	14
	BLA	3	7	3	1	2	10	2	1	10	0	0	13
AEX Resin	IND	0	8	2	1	2	7	1	2	8	2	0	9
	BLA	0	8	1	1	2	6	1	3	6	2	1	7
CEX Resin	IND	0	8	1	0	1	8	0	1	8	2	0	7
	BLA	0	7	0	0	1	7	0	1	7	3	0	4
HIC Resin	IND	1	5	1	0	1	7	0	0	8	0	0	8
	BLA	1	3	1	0	1	4	0	0	5	0	0	5
AEX Membrane	IND	0	5	0	0	2	3	0	0	5	1	0	4
	BLA	0	4	0	0	2	2	0	1	3	1	0	3
CEX Membrane	IND	0	2	0	0	1	1	0	0	2	1	0	1
	BLA	0	2	0	0	1	1	0	1	1	1	0	1
HIC Membrane	IND	0	2	1	0	0	3	0	0	3	0	0	3
	BLA	0	1	1	0	0	2	0	0	2	0	0	2
Mixed Mode Anion Exchange	IND	0	7	0	0	1	6	0	1	6	1	0	6
	BLA	0	4	0	0	1	3	2	1	2	2	0	2
Mixed Mode Cation Exchange	IND	0	4	0	0	0	4	0	1	3	1	0	3
	BLA	0	3	0	0	0	3	1	1	1	1	0	2

Method	Filing Type	Load Conductivity Low	Load Conductivity High	Load Conductivity Not Considered	Wash Volume Low	Wash Volume High	Wash Volume Not Considered	Residence time Low	Residence time High	Residence time Not Considered	Residence time N/A - Run at Linear Velocity
Affinity (Protein A)	IND	0	0	14	5	0	9	1	4	6	3
	BLA	0	0	13	5	0	8	3	4	4	2
AEX Resin	IND	1	2	8	3	0	8	1	0	8	2
	BLA	2	1	7	3	0	6	2	1	5	1
CEX Resin	IND	1	0	8	3	0	6	0	2	4	3
	BLA	3	1	4	3	1	4	1	4	2	1
HIC Resin	IND	0	0	8	3	0	5	0	2	4	2
	BLA	0	0	5	1	0	4	0	1	3	1
AEX Membrane	IND	1	1	3	2	0	3	2	0	2	1
	BLA	1	1	2	2	0	2	2	0	2	0
CEX Membrane	IND	1	0	1	1	0	1	1	0	1	0
	BLA	1	0	1	1	0	1	1	0	1	0
HIC Membrane	IND	0	0	3	0	0	3	0	0	2	1
	BLA	0	0	2	0	0	2	0	0	2	0
Mixed Mode Anion Exchange	IND	1	0	6	2	0	5	0	1	3	3
	BLA	1	0	3	1	0	3	1	0	2	1
Mixed Mode Cation Exchange	IND	1	0	3	2	0	2	0	1	3	0
	BLA	1	0	2	1	0	2	0	0	3	0

Method	Filing Type	Flow rate (load and elution) Low	Flow rate (load and elution) High	Flow rate (load and elution) Not Considered	Flow rate (load and elution) N/A Run at Residence Time	Bed Height Low	Bed Height High	Bed Height Not Considered	Elution pH Low	Elution pH High	Elution pH Not Considered
Affinity (Protein A)	IND	3	0	6	5	2	4	8	3	0	11
	BLA	5	0	5	3	2	4	6	2	0	10
AEX Resin	IND	0	1	8	2	4	0	7	2	0	8
	BLA	1	2	5	1	4	1	3	4	0	4
CEX Resin	IND	2	0	5	2	0	1	8	1	0	8
	BLA	3	2	2	1	1	1	4	1	1	4
HIC Resin	IND	1	0	5	2	0	2	6	0	0	8
	BLA	0	0	4	1	0	1	4	0	0	5
AEX Membrane	IND	0	1	3	1	0	0	5	0	0	5
	BLA	0	2	2	0	1	0	3	1	0	3
CEX Membrane	IND	0	0	2	0	1	0	1	0	0	2
	BLA	0	1	1	0	1	0	1	1	0	1
HIC Membrane	IND	0	0	2	1	0	0	3	0	0	3
	BLA	0	0	2	0	0	0	2	0	0	2

Mixed Mode Anion Exchange	IND	0	0	4	3	0	1	6	1	0	6
	BLA	0	1	2	1	1	0	3	2	0	2
Mixed Mode Cation Exchange	IND	0	0	3	1	0	1	3	1	0	3
	BLA	0	1	2	0	1	0	2	1	0	2

Method	Filing Type	Elution Conductivity Low	Elution Conductivity High	Elution Conductivity Not Considered	Temp Low	Temp High	Temp Not Considered	Collection Criteria Broad	Collection Criteria Narrow	Collection Criteria Not Considered
Affinity (Protein A)	IND	0	0	14	0	0	14	4	0	10
	BLA	0	0	12	0	0	13	6	1	6
AEX Resin	IND	1	1	8	0	0	11	4	0	8
	BLA	1	3	4	0	0	8	7	0	3
CEX Resin	IND	0	1	8	0	0	9	4	0	5
	BLA	0	3	5	0	0	8	6	0	2
HIC Resin	IND	0	1	7	0	0	8	3	0	5
	BLA	0	0	5	0	0	5	3	0	2
AEX Membrane	IND	0	1	4	0	0	5	2	0	3
	BLA	0	1	3	0	0	4	3	0	1
CEX Membrane	IND	0	1	1	0	0	2	1	0	1
	BLA	0	1	1	0	0	2	2	0	0
HIC Membrane	IND	0	0	3	0	0	3	1	0	2
	BLA	0	0	2	0	0	2	1	0	1
Mixed Mode Anion Exchange	IND	0	1	6	0	0	7	4	0	3
	BLA	0	1	3	0	0	4	3	0	1
Mixed Mode Cation Exchange	IND	0	1	3	0	0	4	2	0	1
	BLA	0	1	2	0	0	3	2	0	1

Please add any additional comments regarding Bind and Elute Chromatography including reasons and justification for approach	Typically would not file Protein A capture and/or CEX bind-elute polishing steps due to sufficient clearnce factor by three other steps (e.g. VI, AEX and VF) * * * Have moved away from evaluating VC on Protein A because it is typlically low clearance and variable. * We consider highest loading density as worst-case, but in actuality, given viruses are spiked into load based on total amount (not %), loading theoretically should not matter. We collect process characterization data demonstrating impact of residence time on impurity clearance. If no impact was demonstrated, the risk of impact to viral clearance is low. We use widest possible peak collection, with justification that this would potentially collect more viruses into the product pool. * * * * * Not considered meaning the parameter is set at the process target or is the value of the load material as collected from manufacturing (e.g. pH, conductivity). IND supporting study usually at target parameters (except for load ratio) so not really worst case. * Scale down model considered comparable to production scale if chromatogram profile is similar, step yield is within +/- 10%, and % monomer is +/- 5% of GMP run. Worst case residence time factors in lowest expected flow rate (based on equipment capability at scale) and highest bed height. * * * * * * We consider high loading and wide peak cutting as main worst case criteria by bind-elute chromatography. High load will reduce resolution and wider peak cutting ensures robustness of viral clearance. If we would suspect competition between virus and product binding, we would consider bracket approach for loading (high and low). * * Protein A load density: we routinely test high and low, and we find performance does not change. * * * Worst case scenario in binding mode would be due to 1/ increase binding of virus on the resin 2/ increase elution and collection of virus in elution fraction * * Note: Same parameters for IND and BLA. Some testing has been performed at multiple Resin Load Densities (high and low) though for duplicate runs at least one run is at High. Load was applied as recieved from large scale manufacture, thererfore load concentration, pH and conductivity not adjusted (considered) in the study. CEX: Currently have used gradient elution and collected the whole peak, so specific pH/conductivity values not considered. HIC: Tested with pH gradient elution, so specific pH target not considered. *

Question 3d	Do you use any other bind/ elute chromatography methods? If so, please describe.	Method	Comments
		* * * * * * * * NA * * Cation Exchange or Mixed Mode * * * * * * * * * * * * N/A * *	* * * * * * * * * * Not typically included for viral clearance studies. May consider if total viral clearance for the process is too low * * * * * * * * * * * * * *
		* *	* *

Question 3e	Do you include operational pauses in your bind/ elute chromatography steps?	Yes	No	Comments
		3	12	Pauses for Akta explorer pump washes only * For BLA only * * * * * * * There is no intention to pause during operations * * * * * * * * We typically have short pauses between the chromatography phases (e.g. between load and wash) to change buffers. This is not compared to manufacturing scale and not considered worst case. * * * * * * * Not currently tested *

Question 3f Please indicate the Worst Case Conditions for Flow Through Chromatography

Flow Through Chromatography		Worst Case Condition Parameters Notes: please answer with what you include in your health authority submissions select the appropriate answer from the drop down boxes (Low, High, Not Considered*) *Not considered means that it is not considered necessary as part of the study to define worst case											
Method	Filing Type	Resin Load Density Low	Resin Load Density High	Resin Load Density Not Considered	Resin Load Volume Low	Resin Load Volume High	Resin Load Volume Not Considered	Protein load concentration Low	Protein load concentration High	Protein load concentration Not Considered	Load pH Low	Load pH High	Load pH Not Considered
AEX Resin	IND	0	11	1	0	4	8	1	1	10	3	0	9
	BLA	0	14	0	0	3	11	0	2	12	7	0	7
CEX Resin	IND	0	3	0	0	1	2	0	0	3	1	0	2
	BLA	0	3	0	0	1	2	0	1	2	1	0	2
HIC Resin	IND	0	4	2	0	0	6	0	0	6	0	0	6
	BLA	0	4	1	0	0	5	0	0	5	0	0	5
AEX Membrane	IND	0	6	0	0	3	4	0	2	5	0	0	7
	BLA	0	4	0	0	2	3	0	2	3	0	1	4
CEX Membrane	IND	0	3	0	0	1	2	0	0	3	1	0	2
	BLA	0	2	0	0	0	2	0	1	1	1	0	1
HIC Membrane	IND	0	2	1	0	0	3	0	0	3	0	0	3
	BLA	0	1	1	0	0	2	0	0	2	0	0	2
Mixed Mode Anion Exchange	IND	0	5	1	0	1	5	0	0	6	1	0	5
	BLA	0	5	0	0	1	4	1	0	4	3	0	2
Mixed Mode Cation Exchange	IND	0	2	1	0	0	3	0	0	3	0	0	3
	BLA	0	1	1	0	0	2	0	0	2	0	0	2

Method	Filing Type	Conductivity Low	Conductivity High	Conductivity Not Considered	Wash Volume Low	Wash Volume High	Wash Volume Not Considered	Residence Time Low	Residence Time High	Residence Time Not Considered	Residence Time N/A - Run at Linear Velocity
AEX Resin	IND	0	2	10	0	1	11	4	0	5	3
	BLA	0	7	7	0	3	11	9	0	3	2
CEX Resin	IND	0	1	2	0	1	2	0	0	2	1
	BLA	0	1	2	0	1	2	2	0	1	0
HIC Resin	IND	0	0	6	0	0	6	2	0	2	2
	BLA	0	0	5	0	0	5	2	0	2	1
AEX Membrane	IND	1	1	5	1	0	6	2	0	4	1
	BLA	1	1	3	1	0	4	2	0	3	0
CEX Membrane	IND	1	0	2	1	0	2	1	0	2	0
	BLA	1	0	1	1	0	1	0	0	2	0
HIC Membrane	IND	0	0	3	0	0	3	0	0	2	1
	BLA	0	0	2	0	0	2	0	0	2	0
Mixed Mode Anion Exchange	IND	1	0	5	1	0	5	1	0	2	3
	BLA	1	1	3	1	1	3	2	0	1	2
Mixed Mode Cation Exchange	IND	0	0	3	0	0	3	1	0	2	0
	BLA	0	0	2	0	0	2	0	0	2	0

Method	Filing Type	Flow rate (load and elution) Low	Flow rate (load and elution) High	Flow rate (load and elution) Not Considered	Flow rate (load and elution) N/A - Run at Residence Time	Bed Height Low	Bed Height High	Bed Height Not Considered	Wash pH Low	Wash pH High	Wash pH Not Considered
AEX Resin	IND	0	2	6	4	4	0	8	2	0	10
	BLA	0	6	4	4	6	0	8	5	0	9
CEX Resin	IND	0	0	2	1	1	0	2	1	0	2
	BLA	0	1	1	1	1	0	2	1	0	2
HIC Resin	IND	0	0	3	3	1	0	5	0	0	6
	BLA	0	0	3	2	1	0	4	0	0	5

AEX Membrane	IND	1	0	4	2	0	0	6	1	0	6
	BLA	1	0	3	1	0	1	3	1	0	4
CEX Membrane	IND	1	0	2	0	0	0	3	0	1	2
	BLA	0	1	1	0	1	0	1	0	1	1
HIC Membrane	IND	0	0	2	1	0	0	3	0	0	3
	BLA	0	0	2	0	0	0	2	0	0	2
Mixed Mode Anion Exchange	IND	0	0	3	3	1	0	5	0	1	5
	BLA	0	2	2	1	2	0	3	2	1	2
Mixed Mode Cation Exchange	IND	0	0	2	1	1	0	2	0	0	3
	BLA	0	0	2	0	0	0	2	0	0	2

Method	Filing Type	Wash Conductivity Low	Wash Conductivity High	Wash Conductivity Not Considered	Temp Low	Temp High	Temp Not Considered	Collection Criteria Broad	Collection Criteria Narrow	Collection Criteria Not Considered
AEX Resin	IND	0	2	10	0	2	10	5	0	7
	BLA	0	5	9	0	5	9	8	0	5
CEX Resin	IND	0	1	2	0	1	2	2	0	1
	BLA	0	1	2	0	1	2	3	0	0
HIC Resin	IND	0	0	6	0	0	6	2	0	4
	BLA	0	0	5	0	0	5	2	0	3
AEX Membrane	IND	0	1	6	0	1	6	3	0	3
	BLA	0	1	4	1	1	4	2	0	2
CEX Membrane	IND	0	0	3	0	0	3	3	0	0
	BLA	0	0	2	0	0	2	2	0	0
HIC Membrane	IND	0	0	3	0	0	3	1	0	2
	BLA	0	0	2	0	0	2	1	0	1
Mixed Mode Anion Exchange	IND	0	0	6	0	0	6	4	0	2
	BLA	0	1	4	0	1	4	3	1	1
Mixed Mode Cation Exchange	IND	0	0	3	0	0	3	2	0	1
	BLA	0	0	2	0	0	2	1	0	1

	Please add any additional comments regarding Flow Through Chromatography including reasons and justification for approach	* * * * We consider highest loading density as worst-case, but in actuality, given viruses are spiked into load based on total amount (not %), loading theoretically should not matter. We collect process characterization data demonstrating impact of residence time on impurity clearance. If no impact was demonstrated, the risk of impact to viral clearance is low. We use widest possible peak collection, with justification that this would potentially collect more viruses into the product pool. * * * * * Not considered meaning the parameter is set at the process target or is the value of the load material as collected from manufacturing (e.g. pH, conductivity). * Scale down model considered comparable to production scale if chromatogram profile is similar, step yield is within +/- 10%, and % monomer is +/- 5% of GMP run. Worst case residence time factors in lowest expected flow rate (based on equipment capability at scale) and highest bed height. * * * * * * For Flow-through chromatography, we always consider high load and wide peak cutting as worst case condition. * * Some of these "not considered" could become "high" or "low" if warranted by risk assessment specific to the protein/purification process. That has not happened. * * * Worst case scenario would be due to : 1/ Decreased virus binding to resin 2/ Increased collection of virus in flow through fraction * * Note: AEX BLA selections same as for IND except for those selected. *

		Method	Comments
Question 3g	Do you use any other flow through chromatography methods? If so, please describe.	* * * * * * * * * na * * * * * * * * * * * * * * * N/A * *	* *
		* *	* *

		Yes	No	Comments
Question 3h	Do you include operational pauses in your flow through chromatography steps?	3	11	Pauses for Akta explorer pump washes only * BLA supporting only * * * * * * * There is no intention to pause during operations * * For one project, a pause of 25 minutes after loading was performed for the anion exchange column due to an operational error where loading was stopped. An additional media spike study was performed with a 120 minute pause after loading as a worse case. This was to support viral clearance for an IND filing. Otherwise, not typical * * * * * * We typically have short pauses between the chromatography phases (e.g. between load and wash) to change buffers. This is not compared to manufacturing scale and not considered worst case. * * * * * * * Not currently considered *

Question 3i Please indicate the Worst Case Conditions for Virus Filtration:

Virus Filtration		Worst Case Condition Parameters Notes: please answer with what you include in your health authority submissions select the appropriate answer from the drop down boxes (Low, High, Not Considered*) *Not considered means that it is not considered necessary as part of the study to define worst case											
Method	Filing Type	pH Low	pH High	pH Not Considered	Conductivity Low	Conductivity High	Conductivity Not Considered	Temp Low	Temp High	Temp Not Considered	Flow rate Low	Flow rate High	Flow rate Not Considered
Normal Flow	IND	1	0	14	0	1	14	0	0	15	0	1	14
	BLA	1	0	12	0	1	12	0	0	13	0	1	11
Tangential Flow	IND	1	0	3	0	1	3	0	0	4	0	1	3
	BLA	1	0	3	0	1	3	0	0	4	0	1	3

Method	Filing Type	Membrane load (protein) Low	Membrane load (protein) High	Membrane load (protein) Not Considered	Protein concentration Low	Protein concentration High	Protein concentration Not Considered	Pressure (delta P) Low	Pressure (delta P) High	Pressure (delta P) Both	Recovery Flush volume amount Low	Recovery Flush volume amount High	Recovery Flush volume amount Not Considered
Normal Flow	IND	0	15	0	0	3	12	1	9	5	0	6	9
	BLA	0	13	0	0	3	10	0	5	8	0	9	4
Tangential Flow	IND	0	4	0	0	2	2	1	2	1	0	4	0
	BLA	0	4	0	0	1	3	0	0	4	0	4	0

		%Spike	Total Viral Challenge	Final LVR	Other	Comments
Question 3j	How is total viral load controlled?	8	6	2	0	typically < 1% spike volume * * * Chose the spike based on what we're targeting for a final LRV. * * * * * * Set the target total virus challenge. Spike % depends on virus stock titer. * * * * * * * * * * * * * * To target loadings of >7Log *

		Pressure	Flow	Comments
Question 3k	What is the method for driving flow?	15	1	similar to max operating pressure specified for GMP mfg * * * * * * * * * * * * * * * * * Usually pressure but we started to use constant flow methods as well (not yet submitted). * * * also use pressure when appropriate * * * For Nanofiltration only * *