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An agency of the European Union

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EMA/CHMP/ICH/804363/2022

Overview of comments received

on ICH guideline Q5A (R2) on Viral safety evaluation of biotechnology products derived from cell lines for Human or Animal origin

EMA/CHMP/ICH/804363/2022

Please note that comments will be sent to the ICH Q5A (R2) EWG for consideration in the context of Step 3 of the ICH process.

1. General comments – overview

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Rentschler Biopharma SE	0	0	6	Typically, the individual experiments within a virus clearance study are executed following single virus spike approach. In particular, the removal or inactivation of viruses by a process step is adressed by single virus spike runs. Co- or even multi-virus spikings represent useful alternatives to the standard approach especially in terms of time, resources and material savings. The guideline draft do neither exclude nor include such alternatives. For better guidance we would therefore welcome the inclusion of the alternative approach of multispike into the guideline.	N/A
Rentschler Biopharma SE	0	0	6,1	A growing number of peer-reviewed publications show the value of non-infectious virus surrogates as models for viral clearance. These virus like particles have physical and chemical properties comparable to non-specific « model » viruses (e.g. parvoviruses). In combination with qualified detection assays (e.g. molecular or biochemical) these surrogates allow intensified characterization of process steps dedicated for virus removal. Unlike infectious viruses these surrogates are currently not considered although they could increase understanding of removal mechanisms and thereby lead to higher viral safety.”	Please consider also surrogates adequately reflecting commonly used « model » viruses for use in virus clearance studies.
Rentschler Biopharma SE	0	0	6.6	In addition to the use of empirically determined prior knowledge data as basis to theoretically claim LRV for a given platform process in-silico methods such as mechanistic modelling might be an acceptable sophisticated approach adequately simulating/forecasting virus removal of chromatographic steps, if sufficiently verified. This powerful approach is currently not considered in the guideline draft but reflects future developments in the field.	N/A
Pall Life Sciences	0	0	1	Document currently outlines what data should be submitted in marketing application and registration packages. Could scope be exanded to include clinical material?	Cover regulatory expectations for clinical material

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Lonza	0	0	All	General comment: the terminology of "in vitro-" and "in vivo assays" should be more clearly defined. Table 2 uses "in vivo screening assays" and "in vitro screening assays" which better identify the unspecific nature of the virus screen aside from virus specific screening assays.	Suggestion to use Table 2 wording in Table 1 and throughout the document; maybe adding the definition in the glossary
Lonza	0	0	All	LIVCA and end of production cells; throughout the document can they be defined and consistency applied to avoid confusion.	LIVCA and EPC define both if different in the glossary then apply consistency through the document.
Lonza	0	0	All	A lot of very important information for industry is captured in the appendices and/or the footnotes of tables. For all of these, they should be brought to the main body of the paper and the topic discussed in specific and relevant paragraphs.	Key topics should be moved to the main body of the document and discussed in the relevant sections rather than in the appendices.
Lonza	0	0	All	The whole document implies that viral inactivation is only achieved at low pH, however this is not apparent in all approaches and modalities especially in the way new processes are evolving. Maybe just refer to 'inactivation technologies'	Replace low pH inactivation by low/high pH inactivation or just pH inactivation or just 'inactivation' to avoid being restrictive.
Charles River Laboratories	0	0	General		Suggestion to use "virus clearance" as a collective term for "inactivation" and "removal" instead of "reduction in/of virus infectivity". Also reviewing the whole document on clarity with this respect (e.g. line 867: "removal" should be replaced by either "removal or inactivation" or "clearance")
Charles River Laboratories	0	0	General	The tables (e.g. 1, 4 and A-5) are difficult to read because of the many footnotes. Some footnotes contain further important information which are not detailed in the corresponding chapters (e.g. , Table 1: footnote g and corresponding chapter 3.3.3 or Table 4: footnote 9 and corresponding chapter 5 Case F; etc.)	Suggestion to ensure that important notes of the tables are also addressed in the related chapters. Text will be easier to read tha table footnotes.
Charles River Laboratories	0	0	General	There might be other broad or virus specific methods including non molecular methods emerge in the future; scientifically suitable alternatives to current recommended and advanced molecular methods. This is not considered in this document as the focus is much on new molecular based methods and NGS specifically.	We recommend to add another short chapter under 3.2 (3.2.6) indicating the possibility of other advanced technologies for virus detection and that the suitability should be considered under the same aspects detailed under 3.2.5 as applicable
Charles River Laboratories	0	0	General	Clarifying terms "in vivo", "in vitro", and "effective (with respect to virus clearance)". They are used inconsistently throughout the whole document leading to unclarity or missunderstanding; see related notes lines 197, 211, and 650ff respectively and lines 879ff (chapter 9, glossary)	Should be addressed in chapter 9 - Glossary
EFPIA	0	0	1	Typo: There are two pages labelled "ii". The second half of the TOC should probably be "iii".	
BioPhorum	0	0	All	Industry commends the committee in a more detailed document and further clarification of regulatory expectations with regards to new modalities. However, there is still a big area of unknown with regards to ATMPs, especially cell therapies that use nanofiltration	Add another annex to clarify regulatory expectations with regards to cell therapies (exosomes for example) or clarify whether cell therapies are out of scope and the topic of other guidelines

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BioPhorum	0	0	All	One of the key principles of the document is the assessment of the risk to benefit ratio of performing viral clearance. However, there is no definition for that ratio, how it is expected to be measured, what characteristics are acceptable to include in the discussion, what are not. For example, exposure to low pH typically decreases the yield of a manufacturing process, decreasing the risk to benefit ratio - is this an acceptable justification for not performing viral clearance?	Include more details around the regulatory expectations with regards to the risk to benefit ratio
BioPhorum	0	0	All	General comment: the terminology of "in vitro-" and "in vivo assays" should be more clearly defined. Table 2 uses "in vivo screening assays" and "in vitro screening assays" which better specify the unspecific nature of the virus screen different to virus specific screening assays.	We suggest to use the table 2 wording in table 1 and throughout the whole document; maybe adding the definition in the glossary
BioPhorum	0	0	All	LIVCA and end of production cells - Are they the same? How are they defined? Harmonize throughout the document, confusing at the moment	LIVCA and EPC define both if different in the glossary
BioPhorum	0	0	All	Animal testing, MAP RAP, antibody production assay, antibody production test - consistent throughout, clarify general vs specific tests, they are not interchangeable and need clear definitions - does in vivo include MAP/HAP/RAP assay and the "in vitro cell culture-based" retro virus infectivity assays and other specific cell based assays (like 9CFR)	
BioPhorum	0	0	All	A lot of very important information for industry is captured in the appendixes and/or the footnotes of tables, the BioPhorum highlighted the specifics ones in its comments. For all of these, they should be brought to the main body of the paper and the topic of specifi paragraphs.	
BioPhorum	0	0	All	The whole document implies that viral inactivation is only achieved at low pH, however this is not a reflection of current practices, or of the way new processes are evolving - high and low pH inactivation should be quoted in the document throughout.	Replace low pH inactivation by low/high pH inactivation or just pH inactivation
BioPhorum	0	0	All	The whole document implies that only "Xenotropic Murine Leukemia Virus (XMuLV)" is used and NOT Mo/A-MuLV, howevert this isnot a reflection of current practices, both types are used - "Murine Leukemia Virus (MuLV) should therefore be used throughout the document.	
Parexel International	0	0		Given the GL & its title apply to 'Cell Line' this revision represents a good opportunity to clearly define 'Cell Line' to ensure it's clear to Companies what the guideline does / does not apply to. For example, some companies use cells as components of products (e.g. NK cells). Can it therefore be clarified if the guideline applies to cell therapies or if it only applies to the evaluation of cells used to produce a therapeutic molecule.	
Parexel International	0	0	6.2.3 / 6.2.4	A line similar to line 563 (i.e.; '..in at least two independent studies…') should be added to either of the sections (6.2.3 / 6.2.4) relating to the evaluation of virus removal / clearance much like it has been included in the section on virus inactivation (see line 563)	A line similar to line 563 (i.e.; '..in at least two independent studies…') should be added to either of these sections on the evaluation of virus clearance much like it has been included in the section on virus inactivation (line 563)
PPTA	0	0	table of contents	Add "Tables" after Glossary	See Table of contents, page ii

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Albrecht Gröner	0	0		The revision of ICH Q5A is appreciated, especially the inclusion of genetically-engineered viral vectors and viral vector-derived products, the encouragement to replace in vivo (and, if appropriate, in vitro) studies for adventitious viruses by PCR and/or NGS, continuous manufacturing, and the application of prior knowledge.	
Albrecht Gröner	0	0		The commonly used term in the Guideline "purification step" for removing viruses is inappropriate as, besides chromatography, virus retentive filtration is the dedicated virus removal step and this step is not implemented for protein purification. Protein purification steps as chromatography (or potentially precipitation) contribute to virus removal or may be even effective in virus removal. Nevertheless, purification steps and dedicated virus removal steps should be clearly distinguished (compare e.g., Line 47, 399, 464, (626), 633, 963, 1046, 1155, 1156, 1157, 1168, 1392, 1425)	purification, dedicated virus removal and inactivation steps should be used throughout the guideline
Alliance for Regenerative Medicine	0	2	1. Introduction	The intent of the guideline includes specific considerations for viral safety for Q13 Continuous Manufacturing (CM) and it did not explicitly introduce in the section.	Add reference to guidance Q13 for CM in introduction.
PTC Therapeutics	0	0		Since there are new modalities in the scope, inclusion of more thorough examples where risk assessments are appropriate (e.g. viral clearance not needed) and AAVs examples (since many are manufactured very similarly would be beneficial. For example, an example with hypothetical AAV manufacturing steps which lead to a conclusion on viral control strategy needed. The document should include phase appropriate approach to viral safety evaluation (FIH vs. Registration) and potentially include examples in the Annex of this.	
PTC Therapeutics	0	0		Looking at the ICH Q5A(R2), Section 3, 4, 5, 6 have major changes. A major change is the new test method using NGS for cell line qualification such as testing for MCB, WCB and LIVCA. This new method has been discussed in recent years and it is good to see that the new ICH Q5A version includes it. NGS has great benefit to reduce animal use and testing time, especially for early stage programs which require fast speed to FIH. It facilitates real-time decision-making for virus test for unprocessed bulk or purified bulk as well.	
PTC Therapeutics	0	0		Annex 6 provides some examples for prior knowledge application. Annex 7 gives the guidelines for viral vector products. But it is not clear to me how we can use prior knowledge from known gene products or processes to a new viral vector product for viral clearance evaluation, for example using generic viral clearance package for filing. With more gene products in development phase and market in the future, inclusion of clearer examples of these situations would be beneficial.	

2. Specific comments on text

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
ViruSure GmbH- Andy Bailey	2	9	1	The extent to which ICH Q5A has been applicable to products in clinical development has been a subject of debate for many years. The EU sought to clarify this discussion with the publication of the EMEA/CHMP/BWP/398498/2005 guideline which helped in defining diminished requirements for products in clinical development.	The guideline makes it clear that it outlines the data that should be submitted for marketing authorisation. A comment about data requirements for products in clinical phase might help in avoiding confusion as to potentially diminished requirements for products in clinical development
EFPIA	2	4	1	in current wording Viral Clearance is not covered	Proposed wording "...concerns the evaluation of the viral safety of biotechnology products and their manufacturing processes..."
EFPIA	2	4	1	Minor & Shared Comment : I would change the wording slightly since the viral safety evaluation is the main objective and the testing part is only one consequence of the viral safety evaluation	This guideline concerns the testing and evaluation of the viral safety, including viral clearance and testing, of biotechnology products,
Lonza	3	3	1	What are the regulatory expectations for clinical trial materials? Here Q5A is modified but should an edit of EMA398498/2009 also follow to ensure alignment ? Reference is https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-virus-safety-evaluation-biotechnological-investigational-medicinal- (EMA 398498/2009)	Although reference to this EMEA guideline may not be appropriate in this ICH document, the regulatory expectations from the committee should be clarified for clinical materials / processes. It is assumed that 2 viruses in a VC study is sufficient for all phases of CTA but this is not specifically called out here in this overarching document.
EFPIA	3	4	1	Minor Shared Theme: Application of guideline to CT and/or MA Is the scope applicable to marketing and registration only? Although EMEA 398498 covers early clinical scope a lot of the current provisions for expanded scope are not covered in this 2009 guidance.	Add : principles of document can be applied as guidance for early clinical INDs [whilst EFPIA acknowledge the early draft discussion on this topic indicated the application for marketing authorisation, the team request this question for clarity]
EFPIA	3	4	1	Minor Shared Theme: Application of guideline to CT and/or MA Guideline still limited to market application only. No guidance for clinical trial applications. Reference to corresponding EMA guidelines and state that described approach is accepted?	
BioPhorum	3	3	1	What are the regulatory expectations for clinical trial materials? A commonly used reference in industry is https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-virus-safety-evaluation-biotechnological-investigational-medicinal-products_en.pdf	Reference should be made to this document or regulatory expectations from the commitee should be clarified for clinical materials
EFPIA	4	5	1	Major Consensus Topic : Product Scope Minor: "biotherapeutics and certain biological products..." still sounds confusing. Does the industry have a clear consensus on the definitions of biotherapeutics and biological products and the differences between the two?	Consider more clarification

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Albrecht Gröner	4	4	1	Biotechnology products include biotherapeutics and certain biological products derived from cell cultures ….	Biotechnology products should be defined in the glossary as the title of the guideline refers to products from cell lines of human or animal origin; as inactivated and live attenuated viral vaccines are outside the scope of this document, it should be clearly stated that the biotechnology products covered are based on recombinant DNA technologies. "certain biological products" should be defined, at least some examples should be given (e.g., ...)
EFPIA	6	9	1	Not necessary in intro section. In general, a lot of very topic specific content has been placed in the introduction section, creating a very exhausting and at times confusing introduction. This content would be better placed in the respective sections covering the topic.	Move to glossary
Octapharma Biopharmaceuticals GmbH	8	9	1	Clarify expectation of authorities on TSE safety: is this meaning a need for scientific advice prior submissions?	
EFPIA	10	24	1	Major Consensus Topic : Product Scope Major: There is a potential gap about recombinant live attenuated viral vectors such as Modified Vaccinia Ankara (MVA) and recombinant measles virus. • They are genetically engineered viral vector and should be included in the scope according to Line 14 • Because they are attenuated vaccines, they should be excluded from the scope of the guideline according to Line 23 One could suggest adding the following wording at Line 23: "Genetically-engineered live attenuated viral vaccines are also excluded from the scope of this guidance" This case was not really addressed during the first discussions about the vaccines to be included in the scope. Follow up Note: albeit not in scope due to compatibility with virus clearance, other attenuated viruses may apply in future cases and therefore the comment is for futureproofing	"<<Genetically-engineered>> live attenuated viral vaccines are also excluded from the scope of this guidance" Or restore elements of the Draft 1 revision text such as: "<Additionally, the principles of virus safety outlined in this guideline may be used in conjunction with other guidance>>" [Based on the shared feedback, EFPIA request if these potential conflicting statements in the scope section could be further clarified for futureproofing]

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	10	24	1	Major Consensus Topic : Product Scope Major We welcome the inclusion of viral vectors amenable to viral clearance in the scope. However we would also like to see guidance on how to build a robust viral safety strategy for other types of viral vectors (lentiviral etc), as well as other types of molecules produced in cell lines, that are not amenable to viral clearance.	Could some guidances be provided [EFPIA recognise that the current narrative more stringently limits the scope to new product types that are amenable to virus clearance such that the user of the guideline has clear understanding of the product types requiring application of this guideline. However, as the new product types continue to emerge, the current narrative for the scope may become less clear over time. The team request to clearly outline what products and in and out of scope. The team also request leave open to apply the principles for other product types such that principles could be utilised based on risk assessment even when the product type is not directly in scope. Suggest to at least include the 'principles may be applied'. The team recognise how R2 of the guideline places more emphasis on risk assessment, such that principles of the guidance can be applied (i.e, Even if one aspect not covered, the other aspects could be utilised)]
EFPIA	10	11	1	Original text: "This document covers products produced from in vitro cell culture using recombinant DNA technologies such as interferons, monoclonal antibodies, and recombinant subunit vaccines." Consider streamlining out of date reference to "interferons, monoclonal antibodies, ..." to refer to "proteins, monoclonal antibodies"... We believe specific reference to interferons is an overly specific historical artifact of the timing of the original guideline when rDNA interferons represented a significant portion of approved biotherapeutics and before the proliferation of hundreds of naturally derived and invented recombinant DNA-derived biotherapeutics. Therefore, we recommend replacing "interferons" with "proteins."	Amgen recommends the following revision: "This document covers products produced from in vitro cell culture using recombinant DNA technologies such as interferons proteins, monoclonal antibodies, and recombinant subunit vaccines."
EFPIA	10	24	1	Major Consensus Topic : Product Scope Original text: "Furthermore, the scope includes Adeno-Associated Virus (AAV) gene therapy vectors that depend on helper viruses such as baculovirus, herpes simplex virus or adenovirus for their production. Specific guidance on genetically engineered viral vectors and viral vector-derived products is provided in Annex 7. Inactivated viral vaccines and live attenuated viral vaccines containing self-replicating agents are excluded from the scope of this document." We request that the guideline include examples of 'live attenuated viral vaccines containing self-replicating agents' that are not genetically engineered.	We request that the guideline include examples of 'live attenuated viral vaccines containing self-replicating agents' that are not genetically engineered.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	10	24	1	Major Consensus Topic : Product Scope Neither Annex 7 nor the introduction make it clear if cell therapies are included. While gene therapeutics are specifically called out, for cell therapies, where the viral vector is a starting material, and the process itself does not lend itself to traditional viral clearance steps, it should be made clear if the principles of the guidance are only applicable to the starting material and that the cell therapeutic itself, even though technically considered an ex vivo gene therapeutic in some jurisdiction is not in scope of this guideline.	amend the sentence to state that cell therapies are only in scope as far as the viral vector starting material is concerned.
EFPIA	10	56	1	Major Consensus Topic : Product Scope If we now also include products where the viral vector is the product (e.g. gene therapies) then this scope statement reads incomplete. For a viral vector gene therapeutic viral clearance studies are not really feasible , testing, risk and mitigation strategies are what becomes the focus of viral safety.	Add a statement that speaks to what's in scope for the newly added products e.g. For biotechnology products where the viral vector is the gene therapeutic, this document provides a general framework on testing and risk mitigation based on process inputs. If that's not true, then how is this applicable to these types of products?
Alliance for Regenerative Medicine	11	12	1	Reference recombinant proteins such as growth factors as a product in this section if it applies	Add reference to recombinant proteins following recombinant subunit vaccines.
Alliance for Regenerative Medicine	11	11	1	The use of the term "recombinant subunit vaccines" is not standard vaccine classification nomenclature. Subunit Vaccines include recombinant protein vaccines, VLPs, conjugate and toxoid vaccines. Only recombinant protein vaccine and VLPs would be in the scope of this giudance since conjugate and toxoid vaccines are not made in cells of human or animal origin .	Remove the word subunit and replace with protein.
Alliance for Regenerative Medicine	14	15	1	Whilst ARM understands the focus on AAV with respect to a (small) viral vector capable of undergoing viral clearance steps - we wonder if this is missing a potential opportunity to indicate e.g. via an Annex, the principles (and focus) that could be used (in the absence of viral clearance validation) to mitigate risks from adventitious viruses for larger vector types.	Add Annex to describe principles (and focus) that could be used (in the absence of viral clearance validation) to mitigate risks from adventitious viruses for larger vector types.
Pall Life Sciences	16	22	1	AAV is no longer commonly produced using a helper virus, but via triple transfection or engineered stable producer cell lines. Helper virus processes will become less common and this suggests helper viruses are required for all AAV production. In addition baculoviruses are defined as helper viruses here, but specifically not in the annex. Need to add definition of whether baculovirus expression systems of all types are included in this section and ammend as appropriate. See also comments on line 1333-1334.	"These products may include viral vectors, such as Adeno-Associated Virus (AAV) gene therapy vectors, produced using transient transfection or from a stable cell line. It also includes viral vector-derived recombinant proteins, for example, baculovirus-expressed Virus-Like Particles (VLPs), protein subunits and nanoparticle-based vaccines and therapeutics. Furthermore, the scope includes AAV expression systems that depend on helper viruses such herpes simplex virus or adenovirus for their production."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	18	21	1	Major Consensus Topic: Helper Virus Description/Defintion Major: There are two different uses of baculovirus described in the guidance 1. Baculovirus can be used for the production of Adeno-Associated Virus (AAV) gene therapy vectors [lines 20-21] 2. Baculovirus can be used to produce recombinant proteins in insect cells [lines 18-19] Helper virus (adeno and herpes simplex virus) is classificed as Case F in Line 409-411 Recombinant baculovirus is not classified Proposal to classify both Helper and recombinant baculoviruses in the Case types [Furhermore, while we recognise the current defintion for helper virus in the glossary is aiming to address both uses for baculovirus, EFPIA recognise that since expectations are not clearly specified for protein expression vectors (baculoviruses) as per the shared comments for this consensus topic, it is currently unclear if they fall under Case C or Case F. We believe they should fall under Case C, since there is no evidence of infectivity to humans with baculovirus. Alternatively, if the Case F would be updated to include both helper virus and protein expression vectors, then we suggest that for baculovirus it is sufficient to test 3 purified bulk lots (and not each purified bulk lot) as baculovirus is not infectious to human, and very efficient clearance in the process can been demonstrated. This is the same risk level as Case C and should therefore have the same requirement. This is at least the case for recombinant proteins expressed using baculovirus, for AAV expressed using baculovirus may need risk assessment based on clearance level.]	Specify "Helper baculovirus" in Case F Classify "Recombinant baculovirus" as a Case C [Furthermore, EFPIA consensus proposal to either: 1. Cover protein expression vectors under Case C or 2. Include helper and protein expression vectors under Case F, but clarify in Table 4 footnote 9 and Table A5 footnote f that: Absence of the residual helper virus should be confirmed for each purified bulk. Absence of protein expression vectors such as baculovirus that are not infectious to humans, should be confirmed using data for at least 3 batches.] Note: If covered under Case C, ensure clarity by including Baculovirus as an example within Section 5 (Lines 383-386), and propose to change "no evidence of infectivity to humans" to "lack of evidence for infectivity in humans" If both are to remain covered under Case F, the purified bulk testing expectations have to better delineated, as proposed above]
BioPhorum	19	19	1	Need definition and examples for nanoparticle-based vaccines and therapeutics	To be added to the glossary
Asahi Kasei Bioprocess Europe S.A./N.V.	23	24	1	Oncolytic viruses and other live virus therapies which are not vaccines are not covered in the document scope.	Clarify whether or how the document applies to oncolytic viruses and other potential live virus therapies.
EFPIA	23	24	1	Major Consensus Topic : Product Scope Are cell therapies in scope or excluded ? There does not seem to be provision for cell therapies in lines 10-24.	proposal to add: Cell therapies are also excluded from the scope of this guidance. [EFPIA recognise that cell therapies are out of scope and does not use the nomenclature of gene therapies and therefore there should be no question as to scope in relation to ex vivo gene therapies. However, we recommend to clarify the scope to say that "this includes also viral vectors used to manufacture genetically modified cell therapies]

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Albrecht Gröner	24	24	1	for clarification	Addition … scope of this document, as not produced by recombinant DNA technologies.
EFPIA	28	28	1	How about replacing "To date" with "After more than three decades of wide use". This would be more specific and provide an objective and measurable description of the overall risk.	See column F
Parexel International	28	29	1	The sentence beginning 'To date,' can be considered for removal. This is commentary as opposed to technical guidance.	
EFPIA	32	39	1	Minor Shared Theme: Expand on The Three Principles & Incoporate Risk Assessment Language Major There are not only 3 principle approaches, there are 4.	Proposal to add "Monitoring and clearance of endogenous viruses, if known to be present in a production cell line". [Alternatively, EFPIA suggest adding these detail to the second principle at Line 37]
EFPIA	32	39	1	Minor Shared Theme: Expand on The Three Principles & Incoporate Risk Assessment Language Major: The construct of the revised guideline is not really taking into account the enhanced quality approach, as defined in ICHQ8 to ICH Q14. For this particular topic, the starting point of a viral safety strategy should include a risk assessment, followed by a remediation through viral inactivation/clearance manufacturing steps and validation, and finally the control tests and steps chosen based on a well understood risk.	Add a methodology for viral risk assessment based on ICH Q9. Highlight in the guideline the two approaches (ICH Q8-Appendix 1): - The minimal approach for control test (extensive test package) - The enhanced approach for control test (limited number of tests but based on a well understood risk assessment)
EFPIA	32	39	1	Minor Shared Theme: Expand on The Three Principles & Incoporate Risk Assessment Language In addition to the approaches mentioned, there should be at least mention of a fourth pillar: Process design. This should be interpreted as having appropriate gowning, process closure and segregation throughout the manufacturing process. Reference may be made to ICHQ7.	Insert an additional bullet in list (should probably be bullet point 3 out of now 4, stating: Ensuring adequate process design to protect against carryover of non-virus-reduced material and virus contamination from operators. [Alternatively, EFPIA propose that these aspects for the manufacturing controls could be included at Line 35-36 in ensuring the absence of undesirable infectious viruses]
EFPIA	32	39	1	Minor Shared Theme: Expand on The Three Principles & Incoporate Risk Assessment LanguagePre-treatment of animal-/human-derived raw materials (e.g., gamma irradiation, high temperature treatment, nanofiltration) could be also considered as complementary approach.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Albrecht Gröner	35	39	1	The three principle, complementary approaches should be re-ordered according to the outline of the document: (3) Cell line Qualification: Testing for Viruses; (4) Testing for Viruses in Unprocessed Bulk; (6) Evaluation and Characterisation of Viral Clearance Procedures. This re-ordering of the approaches is in line with the requirement to assess manufacturing steps for virus clearance capacity based on the number of retroviruses / retrovirus-like particles in the unprocessed bulk in order to achieve a sufficiently high margin of virus safety	- Selecting and testing cell lines and other raw materials, including media components, for the absence of undesirable infectious viruses; - Testing the product at appropriate steps of production for the absence of contaminating infectious viruses; and - Assessing the capacity of the production processes to clear infectious viruses.
EFPIA	40	43	1	Not necessary in intro section. In general, a lot of very topic specific content has been placed in the introduction section, creating a very exhausting and at times confusing introduction. This content would be better placed in the respective sections covering the topic.	Move to Annex 7
EFPIA	44	47	1	Not necessary in intro section. In general, a lot of very topic specific content has been placed in the introduction section, creating a very exhausting and at times confusing introduction. This content would be better placed in the respective sections covering the topic.	Move to relevant section [or alternatively EFPIA suggest for better context, to remove the statistical aspect from the beginning of the sentence and bring this line back up under the 3 pilars , after Line 39.]
EFPIA	44	45	1	Minor Editorial: This sentence does not benefit from just throwing the term "statistics" in here without going into details The underlying statistical assumptions require careful consideration of not just the LOD/LOQ & sample size, but also distribution of the data, risk one is willing to take for a false negative etc.	Rephrase to state: A quantitative virus assay's ability to detect low viral concentrations is defined by its limit of quantitation/detection as well as sample size. At the end of the publication, some nice guidance is provided on how to apply statistics, I'd remove it here as it detracts or add a reference to these sections.
EFPIA	45	45	1	Major Consensus Topic: Helper Virus Description/Defintion Here and throughout the document there is a lack of distinction between a viral impurity and contaminant. A viral vector that is used as a helper or to make the product by definition is an impurity, not a contaminant. It would not be considered an adventitious virus, and it's not even an endogeneous virus as it's not just present but rather has been deliberately added to the process to enable manufacture. As such, just like any other process related input, if it's desirable to remove. However, it should be defined as an impurity not a contaminant. We should be very clear on that distinction. If that's too complicated, I'd add this to the glossary, that even though this update now includes products which use viral vectors in the manufacture, we still refer to them as contaminants even though they are technically an impurity.	See comment, Add a definition to the glossary. Alternatively state: Therefore, establishing that an infectious virus contaminant or impurity (this should be done consistently throughout)
EFPIA	45	47	1	Removal or inactivation of viruses not limited to purification process. Could be also pre-treatment of animal-/human-derived raw materials.	EFPIA suggest to remove "purification" and replace with "manufacturing process"?

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	48	56	1	Not necessary in intro section. In general, a lot of very topic specific content has been placed in the introduction section, creating a very exhausting and at times confusing introduction. This content would be better placed in the respective sections covering the topic.	Move to relevant section [or alternatively, EFPIA suggest relocating the new added text Line 40-43 elsewhere will help restore the context of the case-by-case assessment that may apply]
ViruSure GmbH- Andy Bailey	50	54	1	The virus risk from a cell line can be influenced by both the type and species of the cell line and should be considered as one factor that contributes to the virus safety of biotechnology products	The type and species of cell line should also be considered as a key factor of any risk assessment
Asahi Kasei Bioprocess Europe S.A./N.V.	50	54	1	The host cell species is an important consideration to understand which types of viruses can replicate in the bioreactor and whether they pose a threat to human patients.	Include the cell species as a factor to be considered to understand risk and testing strategies. Especially, we reccommend a clear statement regarding risks associated with human cells compared to other species.
EFPIA	54	56	1	Minor. Redudant description regarding virus studies design.	Remove "experiments for assessment of viral clearance"
Albrecht Gröner	62	62	1	for clarification	Addition … used to prevent virus contamination of the finished product
ProPharma Group <Erik Schagen & Kristiena Abbink>	66	68	2	The phrase: "Introduction of ... is discussed in Annex 7" is inserted without proper context. In addition it suggests that Annex 7 discusses the possible introduction of adventitious viruses by the use of MVS or WVS, which is clearly not the main objective of this Annex.	In view of the previous suggestion regarding Annex 7 itself, it is suggested to rephrase this line to "In case of genetically-engineered viral vectors and viral vector-derived products, virus contamination may also arise from the Master Virus Seed (MVS) or Working Virus Seed (WVS)."
EFPIA	68	69	2	Editorial: Avoiding redundancy with Annex 7.	Move Sentence "Use of well characterized banks and MVS or WVS can reduce the risk of virus contamination." to Annex 7
PPTA	68	68	2	Revision of the text see word in red column G	..."Use of well characterised cell banks…."
EFPIA	69	71	2	Original text: “Furthermore, helper viruses used for the production of recombinant proteins, VLPs, or gene therapy viral vector products are also considered as process-related viral contaminants (see Annex 7)." We recommend an editorial revision to state “viral-vector gene therapy products” instead of “gene therapy viral vector products.”	Amgen recommends the following revision: “Furthermore, helper viruses used for the production of recombinant proteins, VLPs, or viral-vector gene therapy viral vector products are also considered as process-related viral contaminants (see Annex 7)."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	69	71	2	Add that this requires demonstration of helper virus clearance?	Since the Line 68-69 speaks to controls for one risk factor for MVS, but not for helper virus clearance, EFPIA propose that the narrative simply outlines these risk factors here or we more systemaically address each control herein
Albrecht Gröner	69	71	2	Helper viruses and gene therapy viral vectors are considered process-related viral contaminants. That's correct, but what is the difference between these viruses and e.g, "intentionally introduced, non-integrated viruses such as Epstein-Barr Virus used to immortalise cell substrates or Bovine papilloma virus" [Glossary, Line 955-956], considered endogenous viruses?	definition of "process-related viral contaminants" to be included in Glossary
Lonza	70	71	2	Helper viruses should not be considered as contaminants, change to impurities. They are part of the process, a better reference is 'process related impurities'	Furthermore, helper viruses used for the production of recombinant proteins, VLPs, or gene therapy viral vector products are also considered as process-related viral impurities. Just refer to process related impurities, as the point is not discussing contamination events.
EFPIA	70	71	2	Major Consensus Topic: Helper Virus Description/Defintion Major: Description of "Helper Virus" as a "Process Related Viral Contaminant". As it is a known and controlled starting material, well tested and characterized, is it really a "Contaminant"?. It is a known and measured entity for which the process is designed to clear and is tracked to suport process control and tested to show process conformance to clear. So i would define as an impurity, not a contaminant. Change all throughout the document.	Proposal to chage line 69 to 71 to : "Furthermore, helper viruses used for the production of recombinant proteins, VLPs, or gene therapy viral vector products are also considered as process-related viral impurity (see Annex 7)." Change all throughout the document. Or provide a glossary on contaminant vs impurity difference.
BioPhorum	70	71	2	Helper viruses should not be considered as contaminants, change to impurities. They are part of the process, a correct qualification is 'process related implurities'	Furthermore, helper viruses used for the production of recombinant proteins, VLPs, or gene therapy viral vector products are also considered as process-related viral impurities
ProPharma Group <Erik Schagen & Kristiena Abbink>	71	71	2	If suggestion (line 31 below), to insert the Annex 7 as Chapter 7, is approved the reference "(see Annex 7)" could be removed.	Remove "(see Annex 7)".
EFPIA	73		2,1	Endogenous virus is more likely than latent/persistent	Propose to change order and start with endogenous retrovirus.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	73	76	2,1	Minor: This seems to ignore that we're now deliberatly introducing viruses into the MCB as well and some of them integrate stably. Given that ~ 50% of the mammalian genome consists of transposons, there should also be a statement that speaks to the risk of potentially generating a new type of virus through recombination events. I think for third generation viral vectors that risk is very low, but we should not assume that we will continue to limit ourselves to these types of vectors.	Add a statement that discusses this risk during generation or propagation of an MCB. [Alternatively, EFPIA suggest the risk factors for the new modalities could be further elaborated within Annex 7, which is dedicated to these product types]
Parexel International	73	74	2,1	Unclear, writing could be improved.	Cell substrates may contain viruses that are latent / pertistent (such as herpesvirus or endogenous retroviruses) that may be transmitted from one cell generation to the next.
EFPIA	75	76	2,1	Editorial: Wording not complete	Proposed rewording "or may become expressed as infectious or defective particles."
Parexel International	75	75	2,1	Consider removing the word 'unexpectedly', it's somewhat subjective / non-technical.	Consider removing the word 'unexpectedly', it's somewhat subjective / non-technical.
EFPIA	77		2,1	Virus word not specific enough as we speak about adventitious viruses.	Proposed rewording: "Adventitious viral contaminants may be introduced:"
Lonza	85	86	2	Helper viruses should not be considered as contaminants as they are part of the process. Perhaps 'impurities' is a better word.	Remove and modify the wording
EFPIA	85	87	2,2	Major: Under the section header of "Adventitious Virus that could be introduced during production", focus on the contamination of the helper virus seed with adventitous agents, and potential Replication Competent Viruses (addition of RCV definition in the glossary-Line 940)	Replace 2) the use of a virus or viral vector (including helper viruses used in their production) to induce expression of specific genes encoding a desired protein (see Annex 7), by 2) the use of contaminated viral seeds (including helper viruses used in their production), including potential contamination by replication competent viruses (RCV) (see Annex 7).
BioPhorum	85	86	2	Helper viruses should not be considered as contaminants as they are part of the process	Remove and nuance wording
ProPharma Group <Erik Schagen & Kristiena Abbink>	87	87	2	If suggestion (line 31 below), to insert the Annex 7 as Chapter 7, is approved the reference "(see Annex 7)" could be removed.	Remove "(see Annex 7)".

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	88	89	2,2	Original text: "[…] such as a monoclonal antibody coupled affinity column resin for product selection or purification;" If an affinity ligand is derived from yeast or E.coli hosts, then concerns for viral safety of the affinity resin would not be present as these hosts are not capable of propagating mammalian viruses.	Amgen recommends the following revision: "[…] such as a monoclonal antibody mammalian derived coupled affinity column resin for product selection or purification;"
ViruSure GmbH- Andy Bailey	89	91	2,2	A potential source of risk is not only the raw materials and medium used during culture, but also other cell lines being handled at the same time. Whilst GMP requirements for cell banking normally exclude this risk, this might be a risk factor early in the history of the cell line	A comment that environmental risks may also arise from other cell lines that are being handled in the same area would be helpful
Asahi Kasei Bioprocess Europe S.A./N.V.	90	91	2,2	There is a specific risk of contaminations occurring from infected operators as many contaminations have been hypothesized to occur by this route.	Specify that contamination from the environment includes contamination by a infected operator.
EFPIA	92	92	2,2	Original text: "Monitoring cell culture parameters can be helpful in the early detection of potential adventitious viral contamination." We believe this text is out of place in the section on introduction of viruses and could be moved to more logical location of monitoring of unprocessed bulk (Section 4).	Amgen recommends moving text to Section 4, for example at line 325, perhaps with slight modification of text in context of the discussion on testing/monitoring: "Monitoring cell culture parameters can also be helpful in the early detection of potential adventitious viral contamination."
Parexel International	92	92	2,2	It would be very useful if guidance / examples could be included of cell culture parameters that can be useful to monitor	Provide examples of these parameters for the cell culture process
Alliance for Regenerative Medicine	92	93	Section 2.2.	Suggests including examples of cell culture parameters where it can be helpful to detect potential contamination early.	Examples of cell culture parameters include XXXX.
EFPIA	95	95	2	Minor: If separating human and animal in one place (e.g., previous sentence), both should be addressed consistently.	Add "human-" in front of "animal-derived raw materials " in line 95, or add a seperate sentence to address "human- raw materials".
ViruSure GmbH- Andy Bailey	97	99	2,2	The point at which testing is performed (i.e. prior to any inactivation step performed on the raw material) is a critical factor and should be mentioned	Any testing for virus contaminants should be performed prior to inactivation wherever possible
Charles River Laboratories	97	97	2.2		suggest to ad: "……..health status of animals (raw material),……."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Pall Life Sciences	100	102	2	When possible, cell culture media or media supplement treatments such as gamma irradiation, virus filtration, high temperature short time processing, or ultraviolet C irradiation can be used as additional virus risk mitigation measures. Recommend adding that whether this is done or not should be based on risk assessment, and also that other methods may be applicable (cover future needs)	When possible, and based on risk assessment, cell culture media or media supplement treatments such as gamma irradiation, virus filtration, high temperature short time processing, or ultraviolet C irradiation can be used as additional virus risk mitigation measures. Other alternative approaches may also be applicable.
Lonza	100	102	2.2	Wording is confusing, this only applies to high risk components, not to all components. Should be clearly linked to the high risk materials (animal derived materials). The proposed wording here implies this is a new expectation for all process components in this paragraph.	'For high risk materials, when possible, cell culture media…' or 'if not applied then sufficient justification provided based upon risk'.
Lonza	100	103	2.2	Industry is moving from gamma irradiation, other inactivating technologies and some of the virus inactivation methods that will be used in the forthcoming years are not defined yet. No reason for this to be an exclusive list so remove the restriction and future proof the document; therefore recommend the wording is less specific.	'For high risk materials, when possible, cell culture media or media supplement treatments such as gamma irradiation, virus filtration, high temperature short time processing, ultraviolet C irradiation or other viral inactivation methods can be used…'
EFPIA	100	102	2	Minor Shared Theme: Clarifications for Media treatment More clarity would be useful here; many will interpret "when possible... can be used..." differently. This is not required or even expected in general, especially for animal-component-free media. These are not all procedures that can be incorporated simply, because they can significantly impact product quality.	Proposed changes: delete "when possible", and add "to address specific virus risks" at the end of the sentence. The proposed new sentence is: " Cell culture media or media supplement treatments such as gamma irradiation, virus filtration, high temperature short time processing, or ultraviolet C irradiation can be used as additional virus risk mitigation measures to address specific virus risks."
EFPIA	100	100	2,2	Minor Shared Theme: Clarifications for Media treatment Major Provide clarity on cell culture and media supplement treatment. Ensure risk-based approach.	Recommend to change "When possible," to "When needed to mitigate risk, ..."
EFPIA	100	102	2,2	Minor & Shared Comment: Clarifications for Media treatment It is important to specify that treatment of cell culture media or media supplements is only recommended when the material has been evaluated to pose a virus risk and a decision to implement such mitigating measures should be done based on a risk assessment. Implementation of virus inactivating/removing treatment of low-risk materials would not add significant benefit to the safety of the product.	When relevant e.g. due to the use of human- and/or animal-derived raw materials, cell culture media or media supplement treatments such as gamma irradiation, virus filtration, high temperature short time processing, or ultraviolet C irradiation can be used as additional virus risk mitigation measures.
BioPhorum	100	102	2.2	Wording is confusing, this only applies to high risk components, not to all components. Should be clearly linked to the high risk materials (animal derived materials).	'For high risk materials, when possible, cell culture media…'

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
BioPhorum	100	102	2.2	Industry is moving from gamma irradiation and some of the virus inactivation methods that will be used in the forthcoming years are not defined yet. Industry therefore recommends that wording is less specific.	'For high risk materials, when possible, cell culture media or media supplement treatments such as gamma irradiation, virus filtration, high temperature short time processing, ultraviolet C irradiation or other viral inactivation methods can be used…'
EFPIA	106	107	3,1	Major Consensus Theme: LIVCA and EoPC Terminology & Definitions Major: To be consistent with other part of the texts, the cells at the limit of in vitro cell age used for production, should always be cells at the limit of in vitro cell age used for production or beyond.	6 3.1 Suggested Virus Tests for Master Cell Bank, Working Cell Bank, and Cells at the Limit of In Vitro Cell Age Used for Production or Beyond
Charles River Laboratories	108	110	3.1	Adding the option to consider "parental cell line" and/or "pre-bank" testing before preparation of MCBs as it could provide additional risk mitigation. It is not mentioned here but the option is defined in footnot g of table 1 and suggests in vivo testing cancellation for MCB if parental cell lines/cell banks were tested by in vivo and/or NGS	Adding an additional chapter before 3.1.1 discussing the option to analyze "parental cell line" and/or "pre-bank" to reduce viral contamination risk in advance of entering the GMP process. See also comment line 879 (chapter 9 gloassary) and suggestion to include definitions of "parental cell line" and "Pre-bank"
EFPIA	108	108	3,1	It might be more convenient to have the Tables where they are discussed, rather than at the end of the document.	Have tables the first time they are discussed instead of having all Tables at the end of the document.
PPTA	109	109		Specify acronym for 'WCB'	Working cell bank (WCB)
ViruSure GmbH- Andy Bailey	111	111	3.1.1	Latent virus infections should not be excluded from the list of potential contaminants	Suggested text: "Extensive screening for endogenous, latent and adventitious viral…."
ViruSure GmbH- Andy Bailey	111	114	3.1.1	Some cell lines carry an exposure risk to more exotic raw materials (e.g. horse serum) and capturing the risk from these exposure risks should form a key part of the MCB risk evaluation	Include a sentence that prior exposure risks may also influence the testing performed on the MCB
EFPIA	111	112	3.1.1	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies State that if host cell line used for production cell line generation was extensively tested and no animal/human-derived materials were used during production cell line generation or MCB generation, testing for adventitious viruses can be limited and indicate in Table 1 which tests are mandatory for MCB irrespective of testing status of host cell line and materials used during generation of the production cell line and MCB.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	113	114	3.1.1	The discussion of highest risk is very traditional biotech focused. Is it really true that the highest risk is for hybrid human/non-human primate hybrids for e.g. a viral vector product? Would this not also benefit from either a more inclusive statement added that speaks to what drives risk for these types of products (e.g. altered tropism/genome/genetic stability) or make this statement more generic to make it generally applicable?	either add an e.g. and give this as one example, or remove example and state "based on risk"
EFPIA	116	117	3.1.1	Major Consensus Topic: Addln Clarity on Need for Suitable NGS Assay Sensitivity In the introduction we hint at the fact that testing has boundaries based on sample size/LOD/LOQ. Without some context around this, especially for an MCB, WCB, EOP where sample size will be limited, where "broad" testing will be hampered by this sample size and the fact that this is unlikely to be a validated assay if it's broad, how much value does this statement provide to the reader without some further context such as e.g examples, references or at least a crossreference to a section where more guidance on this is being provided?	Reference into the section where this is discussed much better. {EFPIA propose a cross refernece to Section 3.2.5 to help address}
ViruSure GmbH- Andy Bailey	117	119	3.1.1	Exposure risks prior to banking should factor in the evaluation	Suggested text: "…….materials of human or animal origin both prior to and during cell line generation and MCB expansion."
EFPIA	119		3.1.1	it is not only MCB expansion, also the freezing medium is important!	Proposal to add "during culturing of parental cells, in addition to cell line generation and banking".
EFPIA	121	121	3.1.2	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies Major: The sentence "Each WCB should be tested for adventitious viruses as described in Table 1" is too much prescriptive, while Table 1 should only be an example given of what testing could look like (as already mentioned in 3.1). The tests for adventitious agents should always be based on a risk assessment which is specfic to the cells, the history of the cells, the culture conditions of the cells, the raw materials, etc.	Replace the sentence: "Each WCB should be tested for adventitious viruses as described in Table 1" By: The testing for infectious adventitious agents must be carried out based on a risk assessment. An example of testing profile is shown in Table 1. [EFPIA agree with this alternative proposal to help address the major theme]
EFPIA	121	122	3.1.2	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies State that if host cell line used for production cell line generation was extensively tested and no animal/human-derived materials were used during production cell line generation or MCB generation, testing for adventitious viruses can be limited and indicate in Table 1 which tests are mandatory for MCB irrespective of testing status of host cell line and materials used during generation of the production cell line and MCB.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	121	124	3.1.2	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies No adventitious virus tests (in vitro, in vivo) for initial WCB if MCB and LIVCA derived from this initial WCB have been tested. What are the test requirements for any subsequent WCB generated? Furthermore, is it mandatory to derive the LIVCA from initial WCB or can it be also derived from a subsequent WCB? In the latter case, a discrimination between test requirements for WCB from which a LIVCA was derived and WCBs from which no LIVCA was derived should be defined. The test requirements for the different WCBs should be also addressed in Table 1.	
EFPIA	122	124	Table 1	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies The decision on which tests are required or may be omitted on the initial WCB is not clear as presented in Table 1. For example, footnote f (lines 996-998) states "The in vitro test is performed on the WCB or on LIVCA cells derived from the WCB".	Clarification requested on how to apply this approach accordingly (ex. prospectively test WCB and LIVCA for well-characterized cell lines such as CHO) and whether the approach applies to initial WCB or all WCB. Potential to add a note about need to test WCB based on risk assessment (ex. use of animal-derived RM or helper virus). [EFPIA agree it is preferable to retain the flexibility to choose which WCB is used for LIVCA. If needed, more clarity as to expectations for WCB testing when LIVCA is not applicable could also be benefical]
EFPIA	124	125	3.1.2	Minor Shared Theme: Why the need to specify that Ab Production Tests are not needed for WCB: "not recommended" or "not expected"/"not necessary" ("not recommended" can imply that it's bad to do)	suggest to change to "not necessary"
EFPIA	124	125	3.1.2	Minor Shared Theme: Why the need to specify that Ab Production Tests are not needed for WCB: Why mentioning specifically antibody production tests as not commended for WCB?	As Table 1, is quite clear, would propose to remove.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	124	125	3.1.2	Minor Shared Theme: Why the need to specify that Ab Production Tests are not needed for WCB: Major: The sentence "Antibody production tests are usually not recommended for the WCB" must be removed: -This is not a section to say which tests have to be carried at this stage or not, Table 1 is already cross-reference in the section for this purpose - Antibody production tests are specific to rodent contaminants, and therefore rodent cell substrate and it is not said - Antibody production tests are tests in animal that can be replaced by molecular methods which should be promoted rather than the antibody production tests (3Rs)	Remove: "Antibody production tests are usually not recommended for the WCB"
EFPIA	124	125	3.1.2	Minor Shared Theme: Why the need to specify that Ab Production Tests are not needed for WCB: Neither the original nor the current update provide the reference to section 3.2.4. where the rationale when there is benefit to this type of test is provided.	Add a reference to section 3.2.4. of this paper
EFPIA	124	125	3.1.2	Minor Shared Theme: Why the need to specify that Ab Production Tests are not needed for WCB: The sentence "Antibody production tests are usually not recommended for the WCB"	Replace the phrase “not recommended” with the more appropriate phrase “not required” or “not necessary”.
ProPharma Group <Erik Schagen & Kristiena Abbink>	124	124	3.1.2	"... similar tests may be omitted on the initial WCB". This phrase suggests that this is not valid for second WCB etc., while Table 1, footnote f states that either the WCB or LIVCA can be tested independent of the number. Possibly this is still a remainder of guideline Q5A (R1)?	Remove the word "initial" to bring in line with Table 1.
Alliance for Regenerative Medicine	124	125	3	Provide explanation of why "Antibody production tests are usually not recommended for the WCB."	Due to XXX reasons, antibody production tests are usually not recommended for the WCB.
EFPIA	125	126	3.1.2	MAJOR: The alternative approach proposed is for testing the WCB in place of the MCB. In this case, the test on the WCB is a surrogate of testing on the MCB. Therefore it does not need to be repeated on each WCB, as mentioned in the revised guideline. Only test recommended on the WCB should be performed on each WCB, and according to the risk assessment.	Replace: An alternative approach in which complete testing is carried out on each WCB rather than on the MCB would also be acceptable By: An alternative approach in which tests recommended only on the MCB are carried out on the first WCB would also be acceptable.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	125	126	3.1.2	Would it be not sufficient to perform the complete testing on one WCB rather than on each WCB? The absence of viruses, apart from expected endogenous viruses, also indirectly proves the virus safety of the MCB.	
Charles River Laboratories	127	137	3.1.3	The definition of LIVCA should be included in the glossary. The glossary line 884 defines EOPC which seems to be the same like LIVCA or the difference is unclear.	Suggest to eliminate EOPC and using LIVCA only or differentiate more clearly in the glossary
EFPIA	127		3.1.3	Major Consensus Theme: LIVCA and EoPC Terminology & Defintions Major Missing LIVCA and 'LIVCA used for production' definition in glossary	Proposal to add LIVCA also in the abbreviations
EFPIA	127	137	3.1.3	Major Consensus Theme: LIVCA and EoPC Terminology & Definitions Major: About Cells at the LIVCA: 1/ It should be clear, that the stage where tests are performed are cells at or beyond the LIVCA. It is not always consistent in the text. 2/ It should be clarified that these cells are also referred as to end of production cells (EOPC), or as cells from an Extended Cell Bank (ECB). WHO TRS978, annex 3 provides clear definitions and differences on EOPC and ECB: *End-of-production cells (EOPCs): cells harvested at or beyond the end of a production (EOP) run.* *In some cases, production cells are expanded under pilot-plant scale or commercial-scale conditions.* *Extended cell bank (ECB): cells cultured from the MCB or WCB and propagated to the proposed in vitro cell age used for production or beyond* . ECB are important when the cells are used for virus production, as it will not be possible to harvest cells at the end of the production run.	3.1.3 Cells at the Limit of In Vitro Cell Age Used for Production The limit of in vitro cell age (LIVCA) established for production should be based on data derived from production cells expanded under pilot plant scale or commercial scale conditions to the proposed *in vitro* cell age or beyond. Generally, the production cells are obtained by expansion of the WCB; the MCB could also be used to prepare the production cells. Cells at the LIVCA or beyond should be evaluated once for those endogenous viruses that may have been undetected in the MCB. Cells at the LIVCA or beyond are also referred to as end of production cells (EOPC) or cells from the Extended Cell Bank (ECB). The performance of suitable tests (as outlined in Table 1) at least once on cells at the LIVCA used for production or beyond would provide further assurance that the production process does not lead to activation of endogenous viruses or amplification of adventitious viruses, including slow-growing viruses. If any adventitious viruses are detected at this stage, the process should be checked carefully to determine the source of the contamination. [EFPIA agree with this alternative proposal to help adderss the major theme]

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	127	137	3.1.3	is it implicity clear that LIVCA is for the detection of endogenous or slow growing adventitious virus in the MCB, WCB or does include the contorl of process to mitigate environmental contamination. Is LIVCA testing linked to the release of the MCB/WCB? For example if a viral contaminant shows up in 1 batch at LIVCA, and cannot be confirmed eitherway as an endogenous virus or a viral contaminant, do we reject and fail the MCB/WCB? Considering LIVCA is not done for late stage clinical the MCB/WCB would already have clinical use. I guess this is why we show viral clearance, but interested to understand the impact and consequence of a failed LIVCA test.	
EFPIA	127	137	3.1.3	Minor Shared Comment: UBH sample matrix types for testing at Table 2 MCB, WCB and LIVCA are in the same chapter; however, it is not clealry stated whether the substrate to be tested for LIVCA determination should be similar to that used for cell bank release ie, cells frozen in the presence of cryoprotectant, or similar to UPB ie frozen as is.	Specify wether EOPC harvested and tested for LIVCA determination should be frozen in the presence or absence of a cryoprotectant prior to testing, should the cells not be tested immediately after beeing harvested.
BioPhorum	127	137	3.1.3	Industry is expecting some recommendations/suggestions for sourcing such cells (collected from a pilot scale, grown small scale using same passage numbers, etc.)	
Lonza	128	132	3.1.3	Add definition for LIVCA and highlight the differences with EOPC - EOPC (under normal production number of generations), is LIVCA beyond the standard / production number of generations. If it is interchangeable, this needs to be defined. If not, this needs to be clarified as testing will be different.	Consistency throughout document.
EFPIA	128	130	3.1.3	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies Major: There are two purpose and goal of the LIVCA testing: endogenous viruses that may have beem undetected in the MCB (line 131-132), and provide further assurance that the production process does not lead to activation of endogenous viruses or amplification of adventitious viruses, including slow growing viruses (line 134-136). The LIVCA test maybe meaningful for genetic stability but does not seem to be meaningful for virus testing. It's a lot of work and cost. For CHO cells, the LIVCA test should be removed due to prior history/experience that LIVCA testing does not provide any new information than MCB testing for retrovirus/endogenous viruses. For adventitious viruses, in vitro testing is done for every batch. In vivo test is not necessary per footnote g in Table 1.	Suggest to add a footnote to Table 1 that retrovirus/endogenous virus testing at LIVCA is not necessary for well characterized cell lines such as CHO based on prior knowledge.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
BioPhorum	128	132	3.1.3	Add definition for LIVCA and highlight the differences with EOPC - EOPC (under normal production number of generations), is LIVCA beyond the normal number of generations. If it is interchangeable, this needs to be defined. If not,this needs to be clarified as testing will be different	Ideally, please define how to perform the phenotypic and genotypic assessments of LIVCA, which attributes are expected to be tested, what scale can be used to generate the starting material to assess LIVCA, whether drug substance quality needs to be assessed, at what scale if required, what attributes are expected to assess cell line purity and bio-safety, what scale can be used to assess genetic stability, are there any expectations to add extra generations, if there are, where do the generations to be accrued for LIVCA samples come from, how are cell samples expected to be collected. Is there an agreement that small scale studies effectively reflect large scale studies?
EFPIA	131	132	3.1.3	Wording 'endogenous' is misleading here (could also be for example slow growing virus)	Proposal to remove "endogenous"
ProPharma Group <Erik Schagen & Kristiena Abbink>	131	132	3.1.3	In the section it is suggested that only once a LIVCA needs to be tested for endogenous viruses. This is not clear from Table 1 or its footnotes. In fact, from Table 1 it may be understood that LIVCA should be tested for each WCB from which it can be derived.	Suggest to align section 3.1.3 and Table 1, by repeating in footnote b, or, if needed, in an additional footnote that for one particular MCB only once a LIVCA needs to be tested for endogenous viruses
Alliance for Regenerative Medicine	131	131	3.1.3	The cell age at the end of production can be variable. In these cases it could be preferable to test the cells beyond the end of production age to cover the variability of end of production cell age.	Add sentence after line 119 "If the age (e.g., population doubling level) of the cell culture shows significant variability at the end of production, then it is recommended to test the cell culture at beyond the proposed in vitro cell age. The time frame could extent to 10% beyond average cell age at end of production."
ViruSure GmbH- Andy Bailey	132	132	3.1.3	Latent virus infections should not be excluded from the list of potential contaminants	Suggested text: "......endogenous or latent viruses that may have been undetected…."
EFPIA	132	132	3.1.3	Major Consensus Theme: LIVCA and EoPC Terminology & Defintions Major "Cells at the LIVCA are also referred to as end of production cells." And in Line 887 "End of production cells are cells at or beyond the LIVCA."– This should be clarified, as a bit contradictory. Cells at the LIVCA are taken from an extended process, reaching the submitted limit of a certain process duration. However, a normal production process is usually shorter than the maximal time submitted and therefore cells at the end of production of a normal process are usually taken much sooner then at LIVCA.	Clear definitions of LIVCA is needed. Is LIVCA a very specific EOPCB sample (i.e. when EOPCB tested for maximun prodyuction length?) or is EOPCB term considered as fully equivalent. Depending the clarification, in other place of the text or table, it would help to write LIVCA/EOPCB instead LIVCA only.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Alliance for Regenerative Medicine	132	133	3.1.3	States "Cells at the LIVCA are also referred to as end of production cells." but the cells used to represent the limit of in vitro cell age may not be end of production cells due, for example, to scientific and/or logistical challenges.	Cells used to define the LIVCA may be end of production cells or other cells grown to an equivalent number of population doublings, passages or similar.
EFPIA	133	134	3.1.3	Statement is to generic. EOP/LIVCA testing for a viral vector or any product where the WCB condiditions create an increased risk of RCR formation and lack of viral clearance opportunities shouldl require testing for each lot as part of release based on risk.	Add a clarifying statement to indicate that the need to only test once would be for well established cell lines/traditional biotech products, but if the risk is higher (e.g. for a gene therapeutic viral vector) testing of each lot would be required. [EFPIA propose that the ongoing clarifications for RCV testing at Annex 7 Table A-5/Table 1 and clearer defintions for LIVCA and EoPC will already address this comment]
ViruSure GmbH- Andy Bailey	135	135	3.1.3	Latent virus infections should not be excluded from the list of potential contaminants	Suggested text: ".....production process does not lead to activation of endogenous or latent viruses or amplification…."
EFPIA	135	136	3.1.3	Better wording for 'activation' needed	Use " reactivation or induction"
EFPIA	136	137	3.1.3	Not only the process should be checked, also MCB and WCB, which may have to be disqualified in the worst case!	Include "checking of MCB/WCB"

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	138	150	3,2	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies MAJOR: ICH Q9 should be considered in this section and a viral risk assessment should be applied as part of the quality risk management of viral safety. The tests to be performed and the steps where the tests should be performed should be defined based on the risk assessment. It should be clear that the risk assessment will allow the addition of test(s) if a risk is identify, but also removal of test(s) if the risk does not exist. Tests that are suggested in Table 1 could be removed if the risk does not exist. It should be mentioned, that when no raw material of animal origins are used from a given stage of production (for example raw material of animal origin were used on pre-MCB but no longer on the next steps), testing could be removed or limited on following steps.	3.2 Recommended Virus Detection and Identification Assays A number of assays can detect endogenous and adventitious viruses. Table 2 lists examples of such assays. These assays are recommended, but the list is not all-inclusive nor prescriptive or definitive. The most appropriate techniques may change with scientific progress; proposals for alternative techniques should be accompanied by adequate supporting data. Manufacturers are encouraged to discuss these alternatives with the appropriate regulatory authorities. A comprehensive testing strategy should be developed following a thorough viral risk assessement, that includes consideration of the cell line origin; the passage history; and the raw materials and reagents used for cell line generation, cell bank preparation, and production (*e.g.* steps that can inactivate or remove viruses). The strategy should include additional or remove assays as appropriate based on risk assessments of the cell substrate, raw materials, and reagents used, and viral inactivation/removal steps. For example, if there is a relatively high possibility of the presence of a particular virus, specific tests or other approaches for detection of that virus should be included unless otherwise justified. On the other hand, if no raw material of animal origin is used after a given step (for example raw material of animal origin not used after the preMCB), limited testing could be performed. Appropriate controls should be included to demonstrate adequate assay sensitivity and specificity.
EFPIA	138	151	3,2	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 MAJOR: Annex 7 - Table A-5 footnote i should be introduced here. It is applicable to all the the cell lines what ever the kind of product it will be used for production.	Add the footnote (Annex 7 - Table A-5 footnote i) content: For cell lines of insect origin tests for species-specific viruses and arboviruses should be carried out. Refer to Table 4 (Case B, C, and E) for action steps to be taken for virus detection in cell substrates used for production.
Alliance for Regenerative Medicine	138	284	3.2	States that head-to-head comparisons with existing methods are not required for introduction of new molecular tests,	So the guidance should clarify what is appropriate to support introduction of a new test (presumably qualification for sensitivity and specificity/range ?)
Alliance for Regenerative Medicine	139	160	3,2	This paragraph requires further guidance in the event that an ad agent nucleic acid is detected, however, infectious virus is undetected. In this scenario the guidance needs to explain if the cell bank is a suitable substrate or not as the case may be.	The guidance needs to provide a recommendation for the scenario of cell being tested nucleic acid positive and infectivity negative

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Parexel International	142	142	3,2	To future-proof the guidance to some degree, a line should be added encouraging Manufacturers to use the most appropriate techniques in line with scientific progress /state-of-the-art.	To future-proof the guidance, a line should be added encouraging Manufacturers to use the most appropriate techniques in line with scientific progress /state-of-the-art.
EFPIA	145	145	3,2	Consider replacing "production" with "manufacturing process" where these different terms are being used to mean the same thing, therefore promoting the clarity of the document.	See column F
ViruSure GmbH- Andy Bailey	149	150	3,2	Matrix interference should be appropriately controlled in any assay	Suggested text: "….demonstrate adequate assay sensitivity, absence of matrix interference and specificity."
Charles River Laboratories	149	150	3.2		Replace the sentence by: Methods should comply with qualification and validation principles and include appropriate controls to demonstrate adequate assay specificity and sensitivity as applicable
EFPIA	149	150	3,2	Major Consensus topic: Alternative to "validation", such as "qualification or validation package" The sensitivity and detection limit might be affected by sample matrix and thus matrix validation is important and should be reflected in the guidance.	Appropriate controls should be included to demonstrate adequate assay sensitivity and specificity. Furthermore each sample type must be verified suitable for analysis.
Lonza	151	152	3.2	NGS uses PCR. For future proofing why not state these assays as 'for example'. Any assay used, perhaps not even developed yet, would be demonstrated fit for purpose as for any product testing assay. Therefore less specific wording on NGS or any other particular assay should be less definitive in terms of the tests discussed in order to future proof the document.	Assays referenced are examples only and should be referred to as such if specifically called out. A protocol or testing strategy describes the assays used whereas the regulation describes what needs to be demonstrated. Other assays and technologies that are on the horizon and will be validated, improved and broader specificity but are not subject to reference in this guide.
EFPIA	151	160	3,2	Minor Shared Theme: Further clarity regarding NGS database Major Guidance on how to handle NGS data is needed. Is there an official data base against which data are compared? Do NGS data be re-compared when updated data base is available? To be defined how NGS data can be used as release assay.	Some guidance to be added.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	151	160	3,2	Major Consensus Topic:Further advocacy to limit application of in vivo testing MAJOR: The *in vivo* assay should no longer be presented as a gold standard as it was historically with the in vitro assay, as shown in the publication of Gombold et al. (2014). Systematic evaluation of in vitro and in vivo adventitious virus assays for the detection of viral contamination of cell banks and biological products. James Gombold, Stephen Karakasidis, Paula Niksa, John Podczasy, Kitti Neumann, James Richardson, Nandini Sane, Renita Johnson-Leva, Valerie Randolph, Jerald Sadoff, Phillip Minor, Alexander Schmidt, Paul Duncan, Rebecca L. Sheets. Vaccines 32 (2014) 2916-2926. https://doi.org/10.1016/j.vaccine.2014.02.021	Next Generation Sequencing (NGS) and Nucleic Acid Amplification Techniques (NATs) such as Polymerase Chain Reaction (PCR) may be appropriate for broad and specific virus detection, respectively. The introduction of these tests may be done without a systematic head-to-head comparison with the currently recommended in vitro and in vivo assays. In particular, a head to-head comparison is not recommended for in vivo assays to meet the intent of the global objective to replace, remove, and refine the use of animals, and supported by the limited performance of In vivo methods for the detection of viral contaminants as shown in Gombold et al (2014). Because of the assay sensitivity and breadth of virus detection, NGS may also be used to replace cell-based infectivity assays, to overcome potential assay limitations, or to detect viruses without visible phenotypes in the assay system. Positive results should be investigated to determine whether detected nucleic acids are associated with an infectious virus.
EFPIA	151	151	3,2	Minor Shared Theme: NGS and HTS terminology Wording when menting Next Generation Sequencing should be updated to ensure understanding. NGS is now more and more refered to High Throuhput Sequencing. Sequencing technologies are evolving the the "next generation" was refering to the Sequencing generation after "Sanger" Method for sequencing. It is more appropraite to refer to HTS for High througput Sequencing since it includes any new sequencing technology that is non specific and broad range	Next Generation High Throughput Sequencing (HTS) and NucleicAcid Amplification Techniques (NATs)such as Polymerase Chain Reaction (PCR) may be appropriate for broad andspecific virus detection [EFPIA discussed how the glossary includes an equivalency statement for NGS, HTS, MPS, and propose to keep the terminology flexible and reflecting the currently consensus nomenclature within EWG and the technology working groups, in recognition that this technology continues to develop]
BioPhorum	151	152	3.2	Doesn't NGS use PCR as well? NGS vs targeted NAT?	
Alliance for Regenerative Medicine	151	151	3.2	Wording "next generation sequencing" - the technology now exists for over a decade	Suggest using terminology such as 'massive parallel sequencing' or 'high-throughput sequencing' instead. Positive results should be investigated using relevant methods such as xxx that are associated with detecting nucleic acids from infectious virus.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Lonza	153	156	3.2	Good to see a head to head not required but not sure this document needs to refer to specific assays here. Regulatory documents are not generally so prescriptive and the reader generally is not looking for a 'protocol' more 'guidance'. The test assay should be demonstrated fit for purpose and that's all that's needed here. Why not state these are example assays and future proof the document.	The document should be less prescriptive in referencing assays suitable for testing. A protocol describes specific methods but a regulatory guideline simply describes what needs to be demonstrated. The reader is not looking for a protocol here just a scope of what needs to be demonstrated. The method just needs to be fit for purpose and qualified / validated as described in analytical testing guidance elsewhere. Specific method references may lead to the document being less future proofed as it may be perceived as restrictive and not inclusive of new methods on the horizon.
BioPhorum	153	156	3.2	Industry is delighted to see that NGS, NAT and PCR mentioned as appropriate methods and that the introduction of these tests may be done without a head to head comparison with the currently recommended in vitro and in vivo assays. However, given the current regulatory feedback on implementation of these technologies, it is highly unlikely that this apporach will be accepted for in vitro assays. What is the intent of the committe to allow this as a global acceptable approach?	Remove
Alliance for Regenerative Medicine	153	160	3,2	Recommendation is non-committal. Specifically in line 153, "The introduction of these tests may be done without a systematic head-to-head comparison…" There is no indication when a head-to-head comparison is needed and when it is not.	Provide additional information to when a comparison of methods is required and when it is not. If there is a general recommendation (e.g. execute a head-to-head testing strategy or back test an existing lot) for first-use of a replacement NGS or NAT method, provide in this section. Suggest e.g. A systematic head-to-head comparison between current and proposed method is not considered necessary if suitable method performance is demonstrated.
EFPIA	154	156	3,2	Minor Shared Theme: Head-to-Head comparison testing and 3Rs Major: extract : "In particular, a head-to-head comparison is not recommended for in vivo assays to meet the intent of the global objective to replace, remove, and refine the use of animals"	Proposition In particular, a head-to-head comparison is not recommended <<requested/ Expected/ Required>> for in vivo assays to meet the intent of the global objective to replace, remove, and refine the use of animals. << information should be provided on the analytical sensitivity of the NGS test sufficiant to ensure the safety of the product in regard to alternative *in vivo* tests>>

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	154	156	3,2	Major Consensus Theme: Addln Clarity on Need for Suitable NGS Assay Sensitivity It's commendable to indicate a head to head comparison is not required, but global acceptance may be hard to implement for manufacturers where some regulatory agencies do still want to see demonstrated comparability/equivalence. A position paper from ICH that would go into more details of using these types of technologies, how equivalence is established without a head to head comparison would be beneficial.	
EFPIA	154	156	3.2	Minor Shared Theme: Head-to-Head comparison testing and 3Rs "not recommended" narrative change proposed	Replace the phrase “not recommended” with the more appropriate phrase “not required” or “not necessary”.
BioPhorum	154	156	3.2	Industry is delighted to see that a head-to-head comparison is not recommended in this context.	
EFPIA	156	156	3,2	Consistency with line 214	"... objective to replace, remove, and refine the use of animal testing.
EFPIA	156	157	3,2	Major Consensus Theme: Addln Clarity on Need for Suitable NGS Assay Sensitivity Major: "Because of the assay sensitivity and breadth of virus detection, NGS may also be used to replace cell-based infectivity assays, to overcome potential assay limitations, or to detect viruses without visible phenotypes in the assay system." Assay sensitivity can vary according to the matrix to be tested. It is not guarantee at this stage that NGS apply directly can have the same senstivity as cell-based assay for certain virus family.	Remove the "Because of the assay sensitivity and breadth of virus detection"
BioPhorum	157	157	3.2	See comments above and below. Was"cell based infectivity assay" used by intention to consider other cell based assay in addition to the "in vitro virus screening assay"? E.g. Retrovirus infectivity assays and specific cell based virus assays like the 9CFR assays for bovine and porcine viruses?	Address through definitions of in vitro and in vivo assays - general terms vs specific assays also need to be clarified

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	158	160	3,2	Major Consensus Theme: Addln Clarity on Need for Suitable NGS Assay Sensitivity Major: extract : Because of the assay sensitivity and breadth of virus detection, NGS may also be used to replace cell-based infectivity assays, to overcome potential assay limitations, or to detect viruses without visible phenotypes in the assay syst	Proposition Because of the assay sensitivity and breadth of virus detection, NGS may also be used to replace cell-based infectivity assays, to overcome potential assay limitations, or to detect viruses without visible phenotypes in the assay system. <<information should be provided on the analytical sensitivity of the NGS test sufficiant to ensure the safety of the product in regard to alternative cell based infectivity assays>>. [EFPIA consider that the suggested additional narrative could be included either at 154-156 or 158-160]
EFPIA	159	160	3,2	Minor comment: "Positive results should be investigated to determine whether detected nucleic acids are associated with an infectious virus" Should we mention the WHO TRS 993 Annex 2 to help answering the question in case of adv agent detected in marketed vaccines?	Refer to a WHO TRS 993 Annex 2 ?
ProPharma Group <Erik Schagen & Kristiena Abbink>	159	160	3.2	"Positive results should be investigated..." versus Table 2 under NGS: "Postitive results ... may require further investigation". It remains unclear if a positive result requires always an investigation. Or does it only depend on the consequence of the positive result, i.e. further testing of MCB is required in case it is not to be discarded (yet).	It is suggested for clarity to align the indicated phrase and Table 2.
EFPIA	165	169	3.2.1	Current text states "… evaluation of particles by Transmission Electron Microscopy (TEM)"	Recommend to include quantification or RVLP by qPCR or Nucleic Acid Amplication Technology (NAT) as described in Section 6.3, lines 630-633.
EFPIA	165	196	3.2.1	This updated version is supposed ot also address gene therapeutics, if so, minimally, this section should start out by indicating how these requirements woudl be applicable to a gene therapy where the cell culture by definition will generate viral particles (as that's the intent) and the focus will be more on ensuring lack of replication competence and/or lack of adventitious viruses. This section needs to include how this would be applicable to a gene therapeutic.	[EFPIA recognise that Annex 7 is to specifically address these product types. An approach with additional cross referencing to Section 3 for cell line qualification may help address this single comment]

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	166	167	3.2.1	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies Major: Test for retroviruses should be carried out for the MCB, but using cells at or beyond the LIVCA. Testing on both the MCB in addition to the cells at the LIVCA and beyond, as proposed in the guideline, would not provide additional safety confidence. It would create a gap with Ph. Eur. 5.2.3.	Tests for retroviruses should be performed for the MCB and for but using cells cultured up to or beyond the LIVCA used for production. [Or alternatively, EFPIA propose that LIVCA could be included within Table 1 as alternative testing location to MCB for retrovirus testing. this ensures the flexibility.]
Lonza	171	172	3.2.1	Suggest to replace "PCR-based RT assay" by the more general term used above: "assay for Reverse transcriptase activity", this will future proof to potential new technologies. It will drive more consistency throughout the document as well.	As in the similar comment on the principle of the document, referring to specific assays may be considered too prescriptive and the point better made on advances in technology by referring to any specific technology as a 'for example' only.
BioPhorum	171	172	3.2.1	Suggest to replace "PCR-based RT assay" by the more general term used above: "assay for Reverse transcriptase activity", this will future proof to potential new technologies. It will drive more consistency throughout the document as well.	
ViruSure GmbH- Andy Bailey	174	174	3.2.1	The term "cross-react" is incorrect in this context	Suggested text: "Because some cellular DNA polymerases can carry an inacte RT-like activity and lead to….."
EFPIA	175	178	3.2.1	Minor Shared Theme: Retrovirus testing for cell lines at Table 1 Detection of viral particles and/or RT activity in cell not expected to produce endogenous retrovirus is to be considered OOS, at least OOE and are to be investigated. However it may not be possible to find a detector cell line, which is permissive.	Propose to change this sentence to: In case retroviral particles and/or RT activity are detected in cell lines not expected to produce endogenous retroviruses such as HEK293, the potential contamination with adventitious retrovirus needs to be thoroughly investigated, including infectivity testing in a permissible cell line if feasible.
EFPIA	175	178	3.2.1	Minor Shared Theme: Retrovirus testing for cell lines at Table 1 Assays should be designed to detect contaminants relevant to the production system. In case of RT activity detection in a rodent MCB, testing for infectious retroviruses should target detection of rodent retroviruses based on the origin of the MCB. By specifying use of a human cell line, rodent retroviruses that do not replicate in human cells may go undetected.	…confirmation of the RT activity (as a result of a retrovirus contamination) or a positive TEM result should be followed by an assay to detect infectious retroviruses in permissible cells, including a cell line supporting replication of retroviruses relevant to the origin of the MCB and a sensitive readout assay for retrovirus detection.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Lonza	176	178	3.2.1	Why is a human cell line specified ? Perhaps replace by 'permissive' cell line as in other references.	Lines 183-186: using relevant permissive cells 183 (e.g., Mus dunni and SC-1 cells for rodent retroviruses) with sensitive readout assays for 184 retrovirus detection (e.g., a product-enhanced Reverse Transcriptase (RT) assay, a Sarcoma-185 Positive, Leukaemia-Negative (S+L-) assay, or an XC plaque assay or a broad molecular assay).
BioPhorum	176	178	3.2.1	Why is a human cell line requested? Replace by permissive cell line	Use wording like in line 183-186: using relevant permissive cells 183 (e.g., Mus dunni and SC-1 cells for rodent retroviruses) with sensitive readout assays for 184 retrovirus detection (e.g., a product-enhanced Reverse Transcriptase (RT) assay, a Sarcoma-185 Positive, Leukemia-Negative (S+L-) assay, or an XC plaque assay or a broad molecular assay).
EFPIA	179		3.2.1	'constitutively' is misleading as endogenous retrovirus may also be inducible.	Delete 'constitutively'
EFPIA	179	181	3.2.1	Minor Shared Theme: Retrovirus testing for cell lines at Table 1 Unclear in which cases a PCR-based RT assay "may not be needed". Omission of testing only in cases where infectivity has been confirmed? Or would testing be "not necessary" irrespective of the result of the infectivity test? If changed, adjust footnote d in line 993 accordingly.	
EFPIA	180	181	3.2.1	Minor Shared Theme: Retrovirus testing for cell lines at Table 1 "may not" is not clear enough	Propose to write "is not"
BioPhorum	180	181	3.2.1	See comment line 171-172	
BioPhorum	180	195	3.2.1	It would be helpful to provide some example methods here for induction studies.	
BioPhorum	180	210	3.2.2	Industry suggests to move the bracketed portion to the end of the sentence, the methods can also replace the cell culture assays	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
BioPhorum	180	230	3.2.4	What is the meaning of "targeted"? Must the targeted molecular method be qualified to address all viruses outlined in table3? Or can agnostic approaches be used as well but coverage of table 3 viruses must still be demonstrated?	Remove targeted. For the animal testing, replace by MAP RAP (antibody production assay)
BioPhorum	180	231	3.2.4	Replace "animal testing" by "antibody production testing" to allow future proofing and for more consistency	
BioPhorum	180	234	3.2.5	Does 'in vivo' include MAP/HAP/RAP assay and the 'in vitro cell culture-based' retro virus infectivity assays and other specific cell based assays (like in 9CF)? There should be a harmonized approach to describe the assays. Different wording is mixed throughout the document, consistency should be implepmented. Calling the same things in different ways throughout the document confuses industry and is likely to confuse regulatory agencies during review.	
BioPhorum	180	239	3.2.5	Industry suggests to remove the sentence 'Targeted NGS methods may also apply for sensitive detection of known viruses' in this chapter which is specific for NATs.	
BioPhorum	180	242	3.2.5.1	Industry would move this sentence under general chapter 3.2.5 as it relates to both NAT and NGS	Replace '(…) can be used to supplement cell culture assays (…)" by '(…) can be used to supplement or replace cell culture assays(…)' to be consitent with earlier wording
BioPhorum	180	249	3.2.5.2	Does 'in vivo' include MAP/HAP/RAP assay and the "in vitro cell culture-based" retro virus infectivity assays and other specific cell based assays (like 9CFR)? See previous comments on an harmonized apporach to describe the assays. Wording is mixed up throughout the document, consistency should be implepmented, otherwise this is confusing.	Moderate wording to accommodate the next few years until NGS is available in a GMP environment
BioPhorum	180	258	3.2.5.2		Replace 'HAP, MAP, an RAP tests and…..' by ' the antibody production test and…..'

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
BioPhorum	180	260	3.2.5.2	What is meant with "harvest"? Is it viral harvest or general harvest from a bioreactor? The following details indicate both, however this should be clearer. NGS can be used to replace in vitro assay only if there is intereference? YES. But this is a limitation which is not aligned with the other sections of the document, other methods may be more appropriate (provided that it is validated and fit for purpose). NGS should be for example, not the direct reference (other methods on the horizon mayy be more appropriate)	Replace 'harvest' by 'product harvest from cell culture'
BioPhorum	180	264	3.2.5.2	Suggest to replace "……..or it can be used to detect viral genome present in particles (viromics)." by "…….or it can be used to detect viral genome present in supernatants or liquids (viromics)." This would be more clear direction, and more consistant.	
BioPhorum	180	265	3.2.5.2	Suggest to replace "……..or it can be used to detect viral genome present in particles (viromics)." by "…….or it can be used to detect viral genome present in supernatants or liquids (viromics)." This would be more clear direction, and more consistant.	Different sections of the document refer to NGS in different manner: the method to use in every situation, or in only specifc cases. Clarification is needed and a consistent story throughout the document. For this paragraph, change to 'Use of NGS can be considered...(259)'
EFPIA	181	182	3.2.1	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies TEM mandatory or can it be omitted for cell lines with a long historical track record (in-house prior knowledge) for the type of retrovirus present (e.g., CHO)? In the latter case, adjust footnote c in line 993 accordingly.	
ViruSure GmbH- Andy Bailey	184	184	3.2.1	SC-1 cells are used only for the detection of ecotropic retroviruses, and are therefore only used to test cells of murine origin. Mus dunni cells on the other hand are used for detection of all types of endogenous rodent retroviruses	Suggested text: "(e.g., Mus dunni and/or SC-1 cells for rodent retroviruses)"
Alliance for Regenerative Medicine	184	185	3	What is considered a sensitive readout level for retrovirus detection when testing cell lines?	with sensitive readout assays for retrovirus detection that can detect xxx levels of virus.
Alliance for Regenerative Medicine	184	185	3		E.g. (Just for a suggestion - using FDA RCR guideline): "For example, with a sensitive readout assays for retrovirus detection that can detect with 95% probability an infectious viral particle if present at a concentration of 1 infectious particle per medicinal product dose equivalent."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	186	186	3.2.1	Minor Shared Theme: Retrovirus testing for cell lines at Table 1 What is the "broad molecular assay" that can be a readout assay for retrovirus infectivity? It's not listed in Table 2 either.	
EFPIA	187	188	3.2.1	Minor Shared Theme: Retrovirus testing for cell lines at Table 1 Major endogenous retroviral particles: all? Or only simple C-types and A-types?	Sentence to be clarified
EFPIA	190	190	3.2.2	Put definition of induction studies in glossary	
Alliance for Regenerative Medicine	190	196	3.2.1	The guidance suggests that induction studies are of limited value. However, if they are required it makes sense to perform the induction studies under actual conditions of manufacture. Induction studies that are not process relevant would be of further limited value.	Add the following sentence after line 196 "However, these induction studies, if necessary, should be conducted by considering actual manufacturing conditions."
Charles River Laboratories	197	197	3.2.2	The term "in vitro" is inconsistently used throughout the documen including tables and footnotest. It is not always clear when "in vitro" refers to general "cell culture based" assays (e.g. some retrovirus assays or the specific 9CFR assay as a specific assay) or the "cell based unspecific virus screening assay" as outlined under this chapter (3.2.2) or "in vitro" is used as a general term to differentiate from in vivo/animal based assays generally and includes molecular and other assays	Suggest to replace the term "in vitro" (and "in vitro for virus screen") by "cell based unspecific screening assay" for the assay described in chapter 3.2.2 and differentiate from "cell based specific screening assays" (retroviruses/bovine-porcine viruses/etc.) and the general term "in vitro assay" intended to differentiate from animal based assays. Other definitions are possible. Recommend definition in the gloassary
EFPIA	197	202	3.2.2	Major: In vitro tests are carried out by inoculating a test article (see Table 2) into various susceptible indicator cell cultures capable of detecting a wide range of human and relevant animal viruses. The choice of cells used in the test should be based on a risk assessment considering the species of origin of the cell substrate to be tested. The panel of cell lines should include a cell line of the species of origin and a human and a non-human primate cell line susceptible to human viruses	Proposal to be added at the end of the section ".... primate cell line susceptible to human viruses. If the cell substrate is of human origin, the panel of cell lines to be tested should include a cell line of the same species of origin, as well as a non-human primate cell line susceptible to human viruses. "
Parexel International	201	202	3.2.2	Consider replacing 'cell line' with 'cell culture' or ''susceptible cells' to avoid any confusion with the cell line used to produce the biotech product.	Consider replacing 'cell line' with 'cell culture' or ''susceptible cells' to avoid any confusion with the cell line used to produce the biotech product.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	202		3.2.2	"A non-human cell line susceptible to human viruses": viral susceptibility is restrictive, as not only human viruses	Proposal "To a wide range of viruses (e.g. Vero cells)"
EFPIA	204	207	3.2.2	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing Major: Update states that for "cell line qualification", a 28d assay should be used. I assume "cell line" refers to both MCB and WCB. For MCB testing this requirement makes perfect sense. However, for WCB the requirement does not seem reasonable: WCBs are manufactured from a MCB under very controlled circumstances, so a 14d assay should suffice.	"For <<MCB>> qualification, the test should be….." [Due to shared feedback, EFPIA request whether it is feasible to provide additional delineation for the testing duration for the various cell lines in scope for this chapter (i.e., parental, MCB, WCB, LIVCA), and propose that at minimum, the clarification should specify MCB, as indicated. For other stages of the manufacturing process, a balanced risk based assessment could be proposed]
EFPIA	204	207	3.2.2	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing Major: Duration of "in vitro assay" not clear in text. Does the term "cell line qualification" refer to MCB testing?	"For <<MCB>> qualification, the test should be….."
EFPIA	204	207	3.2.2	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing The in vitro assay duration is unclear for cells at the LIVCA, but I don't know if we should reopen the topic.	"For <<MCB>> qualification, the test should be….."
EFPIA	204	207	3.2.2	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing Major We think a 28 days In Vitro format and inclusion of both haemadsorpion and haemmaglutination is not a must, at least not for well known cell line (e.g. CHO), whereas it is appropriate for human cell line used for viral vector production.	Some clear guidance is needed when the 28 days In Vitro is needed or not (no for well known rodent cell line like CHO, yes for HEK293 for example), on which cell bank (MCB only) and the extend of end points to be tested (i.e proposal to write haemadsorption and/or haemagglutination)
EFPIA	204	207	3.2.2	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing Please define 'cell line qualification', could mean either cell bank or parental cell line	proposal to change to cell bank qualification

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	204	207	3.2.2	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing Original text: "For cell line qualification, the test should be performed as a 14-day initial cell culture followed by a secondary passage with a 14-day duration followed by observation for both cytopathogenic and hemadsorbing/hemagglutinating viruses." We believe that performing the test for 14 days followed by an additional 14 days is very long and very proscriptive. We suggest using "may" rather than "should" for this recommendation. We also suggest adding text from line 438, which states "When appropriate, a PCR or other molecular method may also be selected as rapid test methods can facilitate real-time decision making."	Amgen recommends the following revision: "For cell line qualification, the test should may be performed as a 14-day initial cell culture followed by a secondary passage with a 14-day duration followed by observation for both cytopathogenic and hemadsorbing/hemagglutinating viruses. When appropriate, a PCR or other molecular method may also be selected as rapid test methods can facilitate real-time decision making."
SGS Vitrology Ltd	204	207	3.2.2	The term "hemadsorbing/hemaglutinating" is unclear. i.e. are tests for both haemadsorbing and haemagglutinating viruses required, or either?	Could the required end-points be clarified, e.g. haemadsorbing and haemagglutinating?
SGS Vitrology Ltd	204	207	3.2.2	Haemagglutination assays are not particularly useful general tests, as quite different assay conditions are required/optimal for haemagglutination by different viruses (including e.g. pH, ion composition, as well as red cell type), and they are not very sensitive, requiring high levels of the haemagglutinin to be present. As these assays use blood, this is also not consistent with a drive to reduce the use of animals in testing. So it would be useful to understand the rationale for this.	Could a rationale for the new requirement for haemagglutination assays be provided?
Alliance for Regenerative Medicine	208	210	3	When would it be applicable to replace a cell culture assay with molecular virus detection methods?	Alternatively, molecular virus detection methods may be used to replace the cell culture assays in these situations xxx.
Alliance for Regenerative Medicine	208	210	3.2.2	Suggest to expand on recommendations toward replacing the cell culture assays, e.g. comparison and/or bridging approach, assay performance criteria, risk assessment expectations etc, or, reference relevant 3.2.X subsections.	
Charles River Laboratories	211	211	3.2.3	Similar to "in vitro", "in vivo" is also not clearly defined and the meaning is unclear throughout the document including tables and footnotes. It's obvious that "in vivo" testing is frequently intended to address the inoculation assay described in this chapter (3.2.3). However, the antibody production test can be regarded an in vivo assay too (chapter 3.2.4). It is not clear when "in vivo" (or" in vivo animal")used in other chapters refers to the assay in chapter 3.2.3 or is used as a general term to cover both animal based assays.	Similar to "in vitro" suggest to check the whole document for the intended meaning of "in vivo" in the content of the relevant chapter 3.2.3 and 3.2.4. Also recommend definition in the glossary

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	211	219	3.2.3	Major Consensus Topic:Further advocacy to limit application of in vivo testing Major Need to perform In vivo not aligned with table 1, when using well known defined media	Adapt wording to clarify when In vivo not needed in alignment with table 1 [EFPIA broadly weclome the narrative included at Table 1 footnote g, and request if these details could be replicated within the main guideline Section 3.2.3 for greater clarity]
EFPIA	211	219	3.2.3	Major Consensus Topic:Further advocacy to limit application of in vivo testing MAJOR: 3.2.3 In Vivo Assays In view of scientific data (see reference below), assays in animal should not be replaced but removed, each time it does not bring added value, compare to other tests proposed as a whole without mentioning that in vivo should be replace by NGS. NGS is not a test designed to replace the in vivo assays per se and it should not be presented as such. This is the risk assessment remediation that should conclude that the test in vivo is of no added value. Reference to the scientific article below should be added: Systematic evaluation of in vitro and in vivo adventitious virus assays for the detection of viral contamination of cell banks and biological products. James Gombold, Stephen Karakasidis, Paula Niksa, John Podczasy, Kitti Neumann, James Richardson, Nandini Sane, Renita Johnson-Leva, Valerie Randolph, Jerald Sadoff, Phillip Minor, Alexander Schmidt, Paul Duncan, Rebecca L. Sheets. Vaccines 32 (2014) 2916-2926. https://doi.org/10.1016/j.vaccine.2014.02.021	Replace: *NGS is encouraged as a replacement for in vivo assays because of the breadth of viruses it detects and because its use promotes the global objective to replace, reduce, and refine the use of animal testing. Use of NGS to replace in vivo assays may be justified by submitting a validation package. Based on risk assessment and on the overall testing strategy, the use of the in vivo assay may include inoculation of test article (see Table 2) into suckling mice, adult mice, and embryonated eggs. Additional animal species may be used depending on the nature and source of the cell lines being tested. The health of the animals should be monitored, and any abnormality should be investigated to establish the cause.* By: Given scientific data showing the poor detection of viral contaminants by *in vivo* assays (include reference Gombold et al) and to promote the global objective to replace, reduce, and refine the use of animal testing (3Rs), the tests in animal should be removed each time it is shown that the tests proposed for adventitious agents, based on the viral risk assessment, shows that the *in vivo* assay does not bring further viral safety assurance. The implementation of in vivo assays for the detection of adventitous agent must be justified to demonstrate the added value of these tests on animal as compared to alternative broad range non specific molecular methods such as NGS/HTS [While EFPIA recognise it may not be possible to include direct literature references, there is broad EFPIA consensus to add suggested narrative as described, to help complement the ongoing initiatives to address 3Rs within the current guideline revision.]

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	211	219	3.2.3	Major Consensus Topic:Further advocacy to limit application of in vivo testing MAJOR: 3.2.3 In Vivo Assays In view of scientific data (see reference below), assays in animal should not be replaced but removed, each time it does not bring added value, compare to other tests proposed as a whole without mentioning that in vivo should be replace by NGS. NGS is not a test designed to replace the in vivo assays per se and it should not be presented as such. This is the risk assessment remediation that should conclude that the test in vivo is of no added value. Reference to the scientific article below should be added: Systematic evaluation of in vitro and in vivo adventitious virus assays for the detection of viral contamination of cell banks and biological products. James Gombold, Stephen Karakasidis, Paula Niksa, John Podczasy, Kitti Neumann, James Richardson, Nandini Sane, Renita Johnson-Leva, Valerie Randolph, Jerald Sadoff, Phillip Minor, Alexander Schmidt, Paul Duncan, Rebecca L. Sheets. Vaccines 32 (2014) 2916-2926. https://doi.org/10.1016/j.vaccine.2014.02.021	
EFPIA	211	219	3.2.3	Major Consensus Topic:Further advocacy to limit application of in vivo testing Include statement comparable to footnote g, that in vivo testing is not necessary for well-characterised cell lines such as CHO, NS0 and SP2/0, based on cell line history; prior knowledge; and other risk-based considerations.	
EFPIA	212	219	3.2.3	Major Consensus topic: Alternative to "validation", such as “qualification or validation package" Two Major comments: - Regarding the sentence "Use of NGS to replace in vivo assays may be justified by submitting a validation package”: Validation data are not always submitted for the testing of starting materials. - We need to ensure that the first strategy with in vivo adventitious agent test is to remove them first, and only replace them by new assay such as NGS when needed. One Minor comment - Proposal to rechape the section by first presenting the in vivo test, and then discuss the alternatives like NGS	- Remove the "Use of NGS to replace in vivo assays may be justified by submitting a validation package” sentence. - Add a first sentence to the section liked with footnote 'g' of the Table 1 - General comments: details in the Table 1 footnote G should be included in the text
Parexel International	212	215	3.2.3	The sentence on NGS should be moved to the end of this section (currently ends on line 219) to improve readability	The sentence on NGS should be moved to the end of this section (currently ends on line 219) to improve readability

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Alliance for Regenerative Medicine	214	215	3	What should be included in this validation package? Could the supplier's validation package suffice?	Use of NGS to replace n vivo assays may be justified by submitting a validation package that includes xxx or can be supported by the supplier's system validation.
EFPIA	215	215	3.2.3	Major Consensus topic: Alternative to "validation", such as "qualification or validation package" "submitting a validation package". Expectation to NGS should be consistent with that for other molecular assays (see line 246)	Prroposed changes: "submitting a qualification or validation package". [EFPIA recognise that additional details are required to support NGS when used to replace traditional assays, however due to commonality of comments received across the NAT methods in general, EFPIA proposed alternative narrative for more consistent application of expectations is suggested here]
Parexel International	215	215	3.2.3	clarify that a CTO can submit a validation package for NGS. They are more likely to develop the method & validate it.	or CTOs
EFPIA	216	217	3.2.3	Needs specification for the 'embryonated eggs' to be used. Industry standard is to use hen's eggs.	Add specification for the 'embryonated eggs' to be 'embryonated hen's eggs'.
Charles River Laboratories	220	229	3.2.4	An antibody productions test is regarded a general method to screen for viral antigens but this chapter focus much on the specidies specific MAP/HAP/RAP assay supplemented by table 3 outlining a list of viruses which can be detected by using this specific assay for rodent derived material. The chapter could be changed to "Virus specific tests" and the MAP/HAP/RAP indicated and described as one out of other potential virus specific assays like PCR (e.g. MVM, Vesivirus for CHO or Sf9 Rhabdovirus) or specific cell based assays screening for specific bovine/porcine viruses (9CFR) or even other viruses.	Suggest to change the title of the chapter to "Virus specific tests" and revise according to the comments made.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	220	231	3.2.4	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies Major We believe that antibody productions tests should not be required for well-known rodent cell lines cultured in chemically defined medium. For well-known rodent cell lines cultured in chemically defined media, MAP and HAP assays are not expected to provide additional diagnostic benefits, as viruses replicating on such production cell cultures have been found to score in the in vitro virus assay (compare e.g. Andrew Kerr and Raymond Nims, Adventitious Viruses Detected in Biopharmaceutical Bulk Harvest Samples over a 10 Year Period, PDA Journal of Pharmaceutical Science and Technology September 2010, 64 (5) 481-485; Gombold J, Karakasidis S, Niksa P, Podczasy J, Neumann K, Richardson J, Sane N, Johnson-Leva R, Randolph V, Sadoff J, Minor P, Schmidt A, Duncan P, Sheets RL. Systematic evaluation of in vitro and in vivo adventitious virus assays for the detection of viral contamination of cell banks and biological products. Vaccine. 2014 May 19;32(24):2916-26. doi: 10.1016/j.vaccine.2014.02.021. Epub 2014 Mar 25. PMID: 24681273; PMCID: PMC4526145.)	Please clarify if MAP/HAP is always needed or can be skipped under some conditions
Alliance for Regenerative Medicine	220	231	3.2.4	When planning to use NGS as a replacement for antibody tests, could validation guidance be provided to compare against other tests?	
EFPIA	221	222	3.2.4	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies Can antibody tests be omitted if host cell line used for production cell line generation was extensively tested and no animal/human-derived materials were used during production cell line generation or MCB generation?	
Parexel International	221	221	3.2.4	This sentence should be elaborated to detail, to what exactly, the potential for exposure exists (for example is it the production system, the materials used, the cell substrate, the cell banks or all of the above?)	This sentence should be elaborated to detail to what exactly the potential for exposure exists (for example is it the production system, the materials used, the cell substrate, the cell banks or all of the above?)

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Charles River Laboratories	228	231	3.2.4	The MAP/HAP/RAP assays is a specific in vivo screening assay with the in principle capability to detect the indicated viruses of table 3. However, it is not requested to demonstrate the capability of the related protocols to detect these viruses (except for the read out assay). In fact, the typical applied protocols of MAP/HAP/RAP assays cannot claim that all these viruses are detected (especially potential variants/strains) It's an in vivo screening assay with reported specifics but no guarantee to detect table 3 viruses even though the read out screens for these viruses specifically. An agnostic molecular based method (NGS) can be regarded equally cable (because of the principle) to detect table 3 viruses and should be regarded a potential replacment for the antibody production assay (like the 3.2.3 in vivo assay).	allowing replacement of the antibody production assay by either targeted or non targeted molecular methods; see also line 1024-1205 (Table 3)
Charles River Laboratories	230	230	3.2.4	What is the meaning of "targeted molecular….." here? Why is it limited to "targeted"? Why isn't an agnostic molecular approach (NGS) equally capable to replace this assay too? See next comment	
EFPIA	230	231	3.2.4	Minor and Shared Comment: Genericise the NAT methods applicable to replace Ab Production Tests Major: extract : Virus-specific PCR or targeted molecular methods can be used as a replacement assay for the animal testing described in Table 3. Targeted (which means that PCR or capture tests) has been deleted and replaced by a reference of the viruses to be detected e.g., include other molecular methods with specificity for viruses in Table 3.	Proposition Virus-specific PCR or targeted <<or other molecular methods that include the panel of viruses described in Table 3>> can be used as a replacement assay for the animal testing described in Table 3. <<information should be provided on the analytical sensitivity of the NGS test sufficiant to ensure the safety of the product in regard to alternative tests>>.
EFPIA	230	231	3.2.4	Major Consensus Topic:Further advocacy to limit application of in vivo testing MAJOR: Antibody Production Tests can also be replaced by NGS. Recommendation to remove these tests done in animals (3Rs)	Replace: *Virus-specific PCR or targeted molecular methods can be used as a replacement assay for the animal testing described in Table 3.* *By* : To promote the global objective to replace, reduce, and refine the use of animals testing (3Rs), it is recommended to replace Antibody Production Tests, when they were needed, by molecular methods (PCR, targeted molecular methods, or NGS). Table 3 provide a list of viruses detected by the Antibody Production Tests.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	230	231	3.2.4	Minor Shared Theme: Genericise the NAT methods applicable to replace Ab Production Tests Section should not limit molecular detection methods to only targeted methods, since non-targeted methods may evolve in time to replace antibody production tests.	Virus specific PCR or other molecular methods (e.g. targeted/nontargeted NGS) can be used as a replacement assay to the animal testing described in Table 3.
Alliance for Regenerative Medicine	232	234	3.2.5	Single-line statement for section summary is non-committal.	Propose pathway or provide context around when a molecular method would be considered supplemental as compared to a replacement to the in vitro or in vivo assays.
EFPIA	233	234	3.2.5	Major Consensus Topic:Further advocacy to limit application of in vivo testing propose to add stronger wording to favor the implementation of alternative to in vivo assay	Molecular methods can be used to supplement or replace in vitro cell culture-based assays and should be used as alternative to in vivo animal assays
EFPIA	233	234	3.2.5	MAJOR: Add a small text under molecular method chapter to remind that detection of nucleic acid sequence is not necesseraly associated with the presence of infectious/live viral contaminant. Propsoe to add the wording of Ph. Eur. 5.2.3 chapter	To Add: "In case of positive results with either broad molecular methods or NAT tests, a follow-up investigation must be conducted to determine whether detected nucleic acids are due to the presence of infectious extraneous agents and/or are known to constitute a risk to human health."
EFPIA	238	238	3.2.5.1	Major comment: Targeted NGS method should be defined as can be related to post-amplification prior NGS, or targeted bioinformatics analysis.	Add details in the definition (line 906) in the glossary
Alliance for Regenerative Medicine	238	239	3.2.5.1	This sentence is effectively covered in section 3.2.5.2 and really has no bearing on the NAT section: "Targeted NGS methods may also apply for sensitive detection of known viruses."	Remove sentence or move to section 3.2.5.2

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	245	246	3.2.5.1	Major Consensus topic: Alternative to "validation", such as “qualification or validation package Not only the "NAT assays should be appropriately qualified or validated for their intended use." Consider adding this sentence as it applies to more analytical strategies and it's not called out in those other places of the document. Specifically, call out for the qualification and the validation as two dimensions of the suitability package.	See column F
EFPIA	245	246	3.2.5.1	Major Consensus topic: Alternative to "validation", such as “qualification or validation package" “appropriately qualified or validated”: Does this imply it would be also sufficient to perform just a qualification of equipment/system? Why different requirement for NAT assays compared to other assays?	
EFPIA	247	247	3.2.5.2	Minor Shared Theme: NGS and HTS terminology Wording when menting Next Generation Sequencing should be updated to ensure understanding. NGS is now more and more refered to High Throughput Sequencing. Sequencing technologies are evolving the the "next generation" was refering to the Sequencing generation after "Sanger" Method for sequencing. It is more appropraite to refer to HTS for High througput Sequencing since it includes any new sequencing technology that is non specific and broad range	3.2.5.2 High Throughput Sequencing (or Next Generation Sequencing)
Alliance for Regenerative Medicine	247	284	3.2.5.2 and elsewhere	use of NGS: We welcome the inclusion of NGS-based methods as potentially acceptable technology, …	...but would be more valuable if included was guidance on use of techniques, bioinformatics and, where relevant, reference databases
EFPIA	248	284	3.2.5.2	Minor Shared Theme: NGS and HTS terminology replace NGS by HTS in the all section	New advanced molecular methodssuch as NGS HTS (also known as Next Generation Sequencing high-throughput sequencing) are available with demonstrated capabilities for broad virus detection. + Replace "NGS" by "HTS" in the all section

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	248	248	3.2.5.2	Minor Shared Theme: NGS and HTS terminology Minor: Regarding the sentence "NGS (also known as high-throughput sequencing)" We should include Massive Parallel Sequencing	Replace by "NGS (also known as high-throughput sequencing [HTS] or Massive Parallel Sequencing [MPS])"
EFPIA	249	250	3.2.5.2	Major Consensus Theme: Addln Clarity on Need for Suitable NGS Assay Sensitivity Major: Assay sensitivity can vary according to the matrix to be tested. It is not guarantee at this stage that NGS apply directly can have the same senstivity as cell-based assay for certain virus family.	Remove "NGS can provide defined sensitivity and breadth of virus detection and can reduce animal use and testing time."
Alliance for Regenerative Medicine	250	284	3.2.5.2	Section references a validation package should be provided for NGS methods. No specific guidance on how to validate an NGS method is found within ICHQ5r2 nor ICHQ2r2. Examples of suitable standards or reference materials for non-specific virus testing are missing. Last line of section encouraging individual conversations with regulatory agencies regarding method validation and data submission effectively goes against the spirit of having a guidance document. This may lead to different expectations for different groups.	Add reference or other recommendation for validation of impurities (limits) assay for assay validation. This could include a list of viruses/classes that would be expected to be included in the validation. Once these minimum expectations are established, consulting with regulatory agencies can be suggested for product-specific testing needs.
EFPIA	251	251	3.2.5.2	Major Consensus topic: Alternative to "validation", such as "qualification or validation package" "validation package should be provided for NGS" although for other assays such as NAT assays (line 246) state "qualified or validated for intended use. "	Change to 'qualification or validation'
EFPIA	252	252	3.2.5.2	Major Consensus topic: Alternative to "validation", such as "qualification or validation package" "method validation": Expectation to NGS should be consistent with that for other molecular assays (see line 246)	Change to 'qualification or validation'
EFPIA	259	262	3.2.5.2	Minor "Use of NGS should be considered particularly for characterisation or testing of a cell substrate and cell bank, for detection of known and unknown viruses, and in a viral seed or harvest if there is assay interference as a result of lack of effective neutralisation of the vector virus (see Annex 7) or toxicity due to the product or media components."	To be rephrased to increase clarity.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Alliance for Regenerative Medicine	259	265	3.2.5.2	The option of using NGS on final product or post-harvest spent media should be mentioned.	
EFPIA	266	267	3.2.5.2	Major: propose to add a small text to mention that selecting the appropriate controls is key for ensuring NGS/HTS method is performed properly on a routine basis	When applying NGSforsensitivedetection of knownvirusesand/orbroad detection ofnovel viruses,applicants should consider several critical steps in the NGS workflow including the selection of appropriate controls for each of these steps.
EFPIA	271	272	3.2.5.2	Minor Shared Theme: Clarity regarding upkeep of NGS Database It is important to establish procedures to use updated versions of the virus database (e.g. for emerging viruses).	...a database with diverse representation of viral sequences of different viral families. Procedures for updating of the database (e.g. for emeraging viruses) must be established. [EFPIA propose this suggested narrative could help address the minor comments for this shared theme]
EFPIA	272	273	3.2.5.2	Omit sentence and include the maximization of virus detection in point 1 (267 – 268) above?	EFPIA propose to merge the sentence 272-273 with 267-268
Alliance for Regenerative Medicine	274	276	3.2.5.2	Examples of suitable standards or reference materials for non-specific virus testing are missing.	At minimum, knowing classes of viruses to be tested to meet expectations of representative mixture of non-specific viral testing would be helpful.
EFPIA	276	276	3.2.5.2	Minor: extract : This can include using currently available reference virus reagents with distinct physical (size, enveloped and non-enveloped), chemical (low, medium, and high resistance), and genomic (DNA, RNA, double- and single-stranded, linear, circular) characteristics to evaluate the performance of the entire NGS workflow or specific steps. It is obvious that the sentence does not concern trancriptomics or genomics, but it is preferable to clarify	Proposition: <<For viromics approaches,>> this can include using currently available reference virus reagents with distinct physical (size, enveloped and non-enveloped), chemical (low, medium, and high resistance), and genomic (DNA, RNA, double- and single-stranded, linear, circular) characteristics to evaluate the performance of the entire NGS workflow or specific steps;
EFPIA	281	282	3.2.5.2	Major: The expression "other standard types" is confusing, because "standard" in this context is an adjective for the noun "types", whereas the noun is intended to be "standard".	Replace with "other types of standard", to be more clear.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	282	284	3.2.5.2	Minor Shared Theme: Reassess the need for discussion with HA for NGS implementation Original text: “Since NGS has a complex workflow, manufacturers are encouraged to have discussions with the appropriate regulatory authorities regarding expectations for method validation and data submission.” We believe the document provides enough information for use of NGS tests. Therefore, an agency meeting should not be an expectation before use of such NGS tests.	We recommend deleting the sentence. [EFPIA recognise that the revision text has helped to clarify the types of discussion that may be required with health authorities when implementing NGS as an alternative method, such as the expected content for the submission. however, over time and with increased application, this expectation is likely to become outdated. Therefore, based on the commonality of comments, EFPIA propose the sentence should be deleted]
EFPIA	282	284	3.2.5.2	Minor Shared Theme: Reassess the need for discussion with HA for NGS implementation Minor: Regarding the sentence 'Since NGS has a complex workflow, manufacturers are encouraged to have discussions with the appropriate regulatory authorities regarding expectations for method validation and data submission.' This sentence should clarify the regulatory authorities to consult.	General comments - the term 'appropriate regulatory authorities' should be clarified in the entire text
EFPIA	282	284	3.2.5.2	Minor Shared theme: Reassess the need for discussion with HA for NGS implementation This is a missed opportunity and will discourage manufacturers from using NGS. If manufacturers truly have to consult with each regulatory agency around acceptance of the approach of NGS for virus detection, then where is the alignment that ICH is supposed to bring forth and that is supposed to help ensure that this data package will be accepted at least by ICH member states in general?	Provide at least a general consensus on what is required for NGS to be acceptable.
EFPIA	282	284	3.2.5.2	Minor Shared Theme: Reassess the need for discussion with HA for NGS implementation 3.2.5.2 Next Generation Sequencing: expectations for NGS method validation and data submission should be defined in the ICH Q5A guideline. Individual discussions of manufacturers with regulatory authorities as proposed in the draft document will hamper introduction of NGS as routine testing method.	
Parexel International	282	282	3.2.5.2	CTOs are more likely to develop NGS methods- make clear that they can meet with regulators	"or CTOs"

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	284	284	3.2.5.2	Major Consensus topic: Alternative to "validation", such as "qualification or validation package" "method validation": Expectation to NGS should be consistent with that for other molecular assays (see line 246)	Change to 'qualification or validation'
EFPIA	285	285	3,3	Would also consider whether there are alternative safe cell lines, which can be used.	
EFPIA	295		4	Major Consensus Topic: Scope, Definition and Sample Matrices of UBH for Conti Manufacturing Major: It has been decided to keep the historical structure of the guideline, which covered recombinant proteins only. With the enlargement of the scope of the guideline, the unprocessed bulk described in this section is specific only to the unprocessed bulk (cell harvest), for the production of recombinant protein in animal cells. Therefore, it is proposed to clarify the title of this section. Another option could be to change the organization of the document, and put this part in appendix, under the same format as the Annex 7.	Replace: *4. TESTING FOR VIRUSES IN UNPROCESSED BULK* By: TESTING FOR VIRUSES IN UNPROCESSED BULK (RECOMBINANT PROTEIN PRODUCED IN ANIMAL CELLS only)
EFPIA	295	334	4	Major Consensus Topic: Scope, Definition and sample matrix of UBH for Conti Manufacturing It would be clear to specify if the scope of the section applies to batch and continuous processing as well as all modalities.	Incorporate text into the section to explicitly state that the scope is inclusive of continuous and batch processing and all modalities within the scope of the document - cross reference Section 1.
EFPIA	295	334	4	Major Consensus Topic: Scope, Definition and Sample Matrices of UBH for Conti Manufacturing lacks critical discussion of representativeness of sampling for e.g.a continuous process with no pooling/distinct batches.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	296	301	4	Original text: "It is recommended that manufacturers develop programs for the ongoing assessment of adventitious viruses in production batches. The scope, extent and frequency of virus testing on the unprocessed bulk should be determined by taking several points into consideration including the nature of the cell lines used to produce the desired products, the results and extent of virus tests performed during the qualification of the cell lines, the cultivation method, raw material and reagent sources and results of viral clearance studies." We believe that if there is vast excess clearance, periodic testing should not be required. Amgen recommends that this text be deleted. (Further contextualisation of the comment: comment applies to the potential to misinterpret the periodiicty for testing endogenous retroviruses, now that the legacy R1 text describing three lots was removed)	Amgen recommends that this passage be deleted. However, if the text is retained, then we suggest the following revision: "It is recommended that manufacturers develop programs for the ongoing assessment of adventitious viruses in production batches. The scope, extent and frequency of virus testing on the unprocessed bulk should be determined by taking several points into consideration including the nature of the cell lines used to produce the desired products, the results and extent of virus tests performed during the qualification of the cell lines, the cultivation method, raw material and reagent sources and results of viral clearance studies if the viral clearance show excess clearance, above and beyond the expected fluctuations of the adventitious viruses present in production batches, periodic testing may not be required." [or alternatively, to ensure Lines 430-433 are sufficiently clear as to more limited periodicity for testing for endogenous viruses at unprocessed bulk now that the detail is removed from Chapter 4]
EFPIA	302	302	4	Major Consensus Topic: Scope, Definition and Sample Matrices of UBH for Conti Manufacturing Text reads "The unprocessed bulk consists of multiple pooled harvests of cells and culture media"	Consider to clarify if this section is for batch and/or continuous manufacturing

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	302	302	4	Major Consensus Topic: Scope, Definition and Sample Matrices of UBH for Conti Manufacturing Original text (line 302): "The unprocessed bulk constitutes one or multiple pooled harvests of cells and culture media." This text doesn't fit the continuous manufacturing paradigm wherein there is not necessarily a "pooled" harvest at any time during production. We suggest adding general language describing that in the absence of a homogeneous pooled harvest (e.g., continuous harvest with connected processing to purification steps), there is no pooled "unprocessed bulk" sample, but testing material from the flow-stream or surge vessel at one or more intervals during production is required and the sampling strategy should be justified.	Amgen recommends adding context upfront and linking to section 7. Amgen recommends the following revision for line 302: "For batch processing, The the unprocessed bulk constitutes one or multiple pooled harvests of cells and culture media." In addition, Amgen recommends the following revision for lines 314-317: "For processes that involve a continuous harvest, there may be no pooled intermediate, and instead the unprocessed bulk sample(s) would be collected from a flow stream or surge vessel. The the sampling strategy (including periodicity and composition of the samples) should be justified because adventitious viruses and endogenous virus particles can variate along the cell culture duration (see Section 7)." [Proposal is also an EFPIA-shared alternative narrative to help address this major theme at EWG]
EFPIA	303	304	4	Major Consensus Topic: Scope, Definition and sample matrix of UBH for Conti Manufacturing Unprocessed bulk testing may not be the optimal for AVA testing - consistent with text for sampling for continuous processing (lines 306-309).	Consider some flexibility in sample selection to ensures that e.g. The clarified harvest could be selected as optimal sample type as well with considerations indicated in lines 309-310.
EFPIA	303	309	4	Major Consensus Topic: Scope, Definition and sample matrices for UBH in Conti MFG Unprocessed bulk testing may not be the optimal for AVA testing - consistent with text for sampling for continuous processing (lines 306-309). Scenario for adherent cell lines on microcarriers should be considered.	Consider some flexibility in sample selection including microcarrier process where the unprocessed bulk would need processing to remove cells from microcarriers or test disrupted cells and supernatant The clarified harvest could be selected as optimal sample type as well with considerations indicated in lines 309-310. [EFPIA agree that the existing narrative Line 306-309 indicating "constitute fluids harvested from the bioreactor" should afford sufficient flexibility for the varied UBH sample types. however, would request that the glossary/narrative is further clarified, through use of more direct language, such as "Where cells are not readily accessible, cell free bulk can be used"]

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	305	306	4	Minor Shared Comment: UBH sample matrix types for testing at Table 2 Major: Extract : Appropriate testing for viruses should be performed on the unprocessed bulk. For perfusion or continuous manufacturing…..	Appropriate testing for viruses should be performed on the unprocessed bulk. <<The most appropriate test article to detect cell viral infection by endogenous or adventitious viruses must be tested. Pending on viral species and cell types, virus particles can be shed in the medium and/or can remain attached to the cell surface, in this case test articles could be a mix of medium and cells for infectivity tests in animals, cell based infectivity assays, genomic PCRs, genomics NGS. The transcriptomic assays requires intact cells to detects viral RNAs synthetized the infected cells; these viral transcripts are biomarkers of cell infection. >> [EFPIA agree that Table 2 addresses the sample types, including UBH matrcies per the glossary defintion. Therefore EFPIA also propose this additional information could be captured within the existing text for test article at Table 2, and use a cross reference from Section 4 to Table 2 using narrative such as "the test article should be appropriate for the applicable assay (see Table 2)"
Parexel International	305	306	3.2.5.2	To strengthen what is meant by 'appropriate testing' the expected qualification of the methods should be expanded upon.	To ensure appropriate testing is performed the manufacturer should perform product-specific qualification of the analytical procedures used for testing unprocessed bulk therby demonstrating they are fit for purpose. This could include an evaluation of the impact of bulk harvest matrix on method performance or a risk-based justification for its absence. It should be detailed how test methods are appropriate for use if method qualification data is being leveraged from other products to establish their suitability.
EFPIA	308	309	4	Minor Shared Comment: UBH sample matrix types for testing at Table 2 Clarity on definition of fluids harvested from the bioreactor	Propose to use "Cell free harvest" (or define fluids in Glossary)
Alliance for Regenerative Medicine	308	309	4	wording is misleading	Rephrase to "In such cases, the unprocessed bulk would be constituted from fluids harvested from the bioreactor."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	309	310	4	Minor and shared comment related to the specificity of progressive filter fouling 'progressive filter fouling' is highly detailed for this context. Should be sufficient to state influence of cell separation technology on the representativeness of these unprocessed bulk...".	Recommend to remove "and progressive filter fouling" [Proposal is also an EFPIA-shared alternative narrative to help address this major theme at EWG]
EFPIA	309	310	4	Minor and shared comment related to the specificity of progressive filter fouling Original text: "The potential influence of cell separation technology and progressive filter fouling on the representativeness of these unprocessed bulk test samples should be considered." This statement implies a virus spike/recovery study may be required using the harvest separation technology, but no further guidance is provided. Amgen is concerned that this statement could lead to requirements for viral challenge/recovery studies using difficult to implement scale-down bioreactor and harvest processes in a viral safety lab setting. This requirement may be ambiguously interpreted by regulators given the nature of the proposed addition to the guideline.	Amgen recommends that this sentence should be removed from the guideline given no guidance is provided and viral challenge/recovery studies are burdensome for biological processes. If this text is retained, further guidance should be provided as to what types of studies could support the suitability of sampling from clarified harvest streams. For example, could "killed" virus recovery suffice?
ViruSure GmbH- Andy Bailey	311	311	4	Some high titre Adenovirus vectors require dilutions as high as 1:1000 before neutralisation becomes feasible, and such a high dilution factor would not be considered as "minimal". It would generally be helpful to provide guidance on the dilution beyond which the ability to detect adentitious virus becomes too compromised	Please provide clarification to the meaning of "minimal sample dilution"
Charles River Laboratories	312	314	4	the wording is a bit unclear	suggestion: In certain instances, it may be appropriate to test both intact cells and cell lysates (mixture of disrupted cells and related cell culture supernatant)

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	312	314	4	Minor Shared Comment: UBH sample matrix types for testing at Table 2 Major: Need clarification on if it is appropriate to use disrupted cells as unprocessed bulk for the case where the product is recovered or released by cell lysis.	proposal to modify (line 302 to 303): "The unprocessed bulk constitutes one or multiple pooled harvests of cells and culture media. A representative sample of the unprocessed bulk, [...]." By: "The unprocessed bulk constitutes one or multiple pooled harvests of cells and culture media. When intact cells are not available due to cell lysis occuring during production, the unprocessed bulk would constitute fluids harvested from the bioreactor. A representative sample of the unprocessed bulk, [...]" Additionally, proposale to modify line 313: "may be more appropriate to test a mixture of both intact and disrupted cells and their cell culture" by "may be more appropriate to test a mixture of both intact and disrupted cells (if available) and their cell culture"
EFPIA	312	312	4	Minor Shared Comment: UBH sample matrix types for testing at Table 2 *Major:* *"In certain instances, it may be more appropriate to test a mixture of both intact and disrupted cells and their cell culture supernatants that were removed from the production reactor before processing"*: " In certain instances" is imprecise.	"In certain instances" should be clarified
EFPIA	314	317	4	Major Consensus Theme: Periodicity for Sampling in Conti Manufacturing Major: …" due to the potential for adventitious viruses …. To variate along the cell culture duration". Current practice is to test long-term cultivations at close-down, releasing the entire cultivation. If the intention is to discontinue current practice, more guidance should be given as to how frequent testing is required (daily, weekly, biweekly ?). This section is not aligned with the chapter on CM, which only states that RVLP could vary during production period – see lines 827 - 830. This is well established.	….For processes that involve continuous harvest, the sampling strategy (including periodicity and composition of the samples) should be justified, due to the potential for adventitious viruses and endogenous virus like particles to variate along the cell culture duration…. [Proposal is also an EFPIA-shared alternative narrative to help address this major theme at EWG]

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	314	317	4	Major Consensus Theme: Periodicity for Sampling in Conti Manufacturing Text reads ” due to the potential for adventitious viruses …. To variate along the cell culture duration”. However, the guidance does not specify the duration for which the testing will need to be conducted (ex. number of lots) and is broader than the RVLP testing requirement for batch processing in that it includes testing for adventitious agents. To be consistent with the expectation for batch processing, it is recommended to specify or cross-reference the guidance for batch processing.	To be consistent with the expectation for batch processing, it is recommended to specify or cross-reference the guidance for batch processing and should be limited solely to RVLP testing (either by TEM or qPCR). Reference Section 5, Cases B, C and D, Lines 370-403.
EFPIA	316	317	4	Major Consensus Theme: Periodicity for Sampling in Conti Manufacturing "…because adventitious and endogenous virus particles can variate along the cell culture duration." Variation of RVLP is already fully addressed in section 7.2.1 and needs not be addressed here also. Adventitious virus contaminatin in continuous cultivation processes (perfusion systems) is addressed in lines 330-334, and also needs not be addressed here.	"… the sampling strategy should be justified." Delete ".. Because adventitious ….. Section 7)"
Alliance for Regenerative Medicine	316	317	4	The wording is unclear: "… because adventitious viruses and endogenous virus particles can variate along the cell culture duration"	Reword "variate along the cell culture duration" to clarify the intended meaning.
EFPIA	318	325	4	Major Consensus Topic: More clarity on options to substitute IVV with targeted NAT •Q5A, as a general guidance, should leave room for the industry to use evolving science and knowledge to justify approaches with appropriate methods (e.g. currently there is an industry-wide effort to assess the virus risk of CHO cell lines and modernize CHO virus safety testing including using targeted NGS for in-process testing). •For example, targeted NGS, by enriching for viral sequences before sequencing, can be significantly better than the shotgun NGS in the sequencing parameters (e.g. more sensitive with hundreds to thousands of fold-increase in viral reads, significant increase in the percentage of viral sequence coverage, identification of divergent viral sequences by hybrid-capture method), resulting in detecting even more (compared to shotgun NGS method) spiked viruses in several publications. •Targeted NGS can have faster turnaround time. Certain products and processes (eg. continuous manufacturing) may require or benefit from fast assay turn around time.	Add to this section: <<With scientific progress and accumulation of prior knowledge, other methods, eg. targeted NGS might be considered if justified by risk assessment of the cell line and production process, and assay qualification (sensitivity, specificity, and speed). An example is, for well-characterized cell lines such as CHO cells, targeted NGS can be considered if sufficient qualification of the assay (e.g., on assay sensitivity and specificity) is provided.>> or [EFPIA suggest to Replace "broad" with "agnostic" or "unbiased" for NGS detection throughout the document, and define both in NGS defintion in glossary.]

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Biosimilar Medicines Group - Medicines for Europe	318	318	4	States that adventitious agent testing should be applied routinely for every batch but only data from minumum 3 is required for submission (lines 431/432 and 1041)	Clarify the expectation for this testing since it could be misleading and lead to some Companies only testing 3 batches and others testing all.
EFPIA	320	322	4	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing Text reads "Based on risk assessment (including the cell substrate, use of animal-derived raw materials or reagents, and the level of virus in the process, the indicator cell culture should be observed for at least 2 weeks. The language is not clear as to what level of risk warrants a 2 weeks observation and reads like a uniform requirement, or when to do great/less than a 2 week observation.	Clarification requested on risk assessment and level of risk (ex. specific examples) that would warrant a two week observation. [EFPIA agree with the existing structure of this inserted narrative, and which reflects the scope of the products for this chapter. If additional risk assessment details were required in this sentence, suggest to further emphasise on the risk based application for well-characterised substrates. Expectation needs to be balanced for the product types in scope]
EFPIA	320	322	4	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing Original text: “Based on the risk assessment […], the indicator cell cultures should be observed for at least 2 weeks” The insertion of the specific language about observation period seems out of place in the section. The linkage between the observation period and the risk assessment is not clear. Would a shorter observation be warranted with a different risk assessment, or is the observation period merely based on sensitivity of the tests?	if the sentence is retained, the guideline should provide more text explaining the relevance of the observation period to the risk assessment.
EFPIA	320	322	4	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing What is considered to be an adequate level of clearance to allow for a minimum observation time of 2 weeks?	
SGS Vitrology Ltd	321	322	4	For in vitro cell culture infectivity assays performed on unprocessed bulk harvest, assay end-points are not mentioned - is there also an expectation that in addition to observation for cytopathology, haemadsorption and haemagglutination end-points are included (as in Section 3.2.2). Could this be clarified?	Could the required end-points be clarified, e.g. cpe only or cpe and tests for haemadsorbing and haemagglutinating viruses?

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	322	322	4	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing Major What about the necessity to perform haemadsorption/ haemagglutionation at the end of the 2weeks incubation period	Proposal to indicate that haemdsorption OR haemaglutination is needed
EFPIA	326	327	4	Minor and Shared Comment:Forward processing of UBH Major Sentence does not reflect current practice as cell testing takes longer than forward processing of unprocessed bulk harvest. We should not need to wait for test result before processing the bulk harvest	We propose to write "If any adventitious viruses are detected at the unprocessed bulk the product cannot be released and approritate measures decontaminate facility need to be taken". [EFPIA agree that minor additional context be provided, such as "results are not readily available at the time of forward processing"]
EFPIA	326	327	4	Minor and Shared Comment:Forward processing of UBH “harvest should not be used for product manufacture” implies that purification should not be started but this is impossible due to assay time.	Change to “product should not (or cannot) be released”
EFPIA	330	330	4	Major Consensus Topic: Scope, Definition and Sample Matrices of UBH for Conti Manufacturing Put definition of "sublot" in glossary	
Alliance for Regenerative Medicine	330	334	4	States “For continuous manufacturing processes, release of a final sublot requires documented absence of viral contamination for the period during which cultivation fluids were harvested for manufacture of that sublot.”	Include guidance for what samples should therefore be tested: for example, does this mean testing at least samples at the beginning and end of harvesting ?
EFPIA	331	331	4	Minor and Shared Comment:Forward processing of UBH "requires documented absense of viral contamination for the period...". Consider same caveat in line 327 "unless justified" as this should apply equally to continuous and batch processing methods.	add "unless justified".
EFPIA	335		5	Major For the section, Why is there so much focus on A-particles? The relevant particles are the C-types!	Please clarify.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	335	411	5	The section and discussion completely omits gene therapies which are supposed to be included. Minimally, it should be stated, what, if anything, is applicable to gene therapies. Ideally, a case scenario for gene therapies would also be included.	
Albrecht Gröner	335	336	5	purified bulk has to be defined in Glossary; purified bulk represents the drug substance at the end of the down-stream process ?	Definition of "Purified Bulk" in Glossary
Octapharma Biopharmaceuticals GmbH	346	348	5	Please specify the approach to assess the potential virus load in bulk; e.g. case A cells where no specific virus can be assumed. Is there a default assumption suggested by authorities?	If case A is not intended here, please specifcally exclude this scenario.
EFPIA	348	349	5	"helpful" word not adequate	'necessary' instead of 'helpful'?
PPTA	348	350	5	Time course studies are less relevant to removal steps - explained more clearly in lines 437 and 438	
EFPIA	349	352	5	Major consensus topic: Prior knowledge Since prior knowledge can replace a product specific virus clearance study, it should be mentioned here to be consistent with the subsequent discussion (6.6 and Annex).	"When evaluating clearance of known contaminants, in-depth time- dependent inactivation studies, demonstration of reproducibility of inactivation or removal, and evaluation of process parameters should be performed., as applicable."
EFPIA	349	352	5	Current wording implies that requested criteria are only for known contaminants.	
EFPIA	354		5	Please define: What are characterization studies? Virus clearance validation studies?	Proposal to remove 'in characterization', to leave only viral clearance studies

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	361	361	5	Minor, Editorial : Proposal “The most common cases are Case A, B and F.”	Proposal “The most common cases are Case A, B <<and F>>.”
EFPIA	363	364	5	Major Consensus Topic: Helper Virus Description/Defintion Minor: It seems that Class F should be also included in "Cases C, D or E" and "Cases C, D and E).	
EFPIA	365		5	inactive or remove wording' not adequate	‘and/or’ instead of ‘or’?
ViruSure GmbH- Andy Bailey	367	369	5	There are CHO products where no retrovirus like particles were detected in the cell bank or unprocessed bulk harvest, and where also the RT-test was negative. It is known though that CHO cells carry endogenous retrovirus elements, even if they are not expressed at high levels. Given the low sensitivity of tests like TEM, is it sufficient to conclude that there is no retrovirus present? Most authorities would I think still request studies with a retrovirus model virus.	In Case A, If no retrovirus like particles are detected in a CHO derived cell line, would studies with a retrovirus model virus therefore not be required?
EFPIA	367	411	Section 5	EFPIA consensus minor comment Class C through D "Time-dependent inactivation for identified (or “relevant” or specific “model”) viruses at the critical inactivation steps should be obtained as part of the process evaluation for these viruses. Purified bulk should be tested using suitable methods with high specificity and sensitivity for detecting the virus in question. For the purpose of marketing authorisation, data from at least 3 lots of purified bulk manufactured at pilot plant scale or commercial scale should be provided." applies to all classes.	For Class C and D suggest to delete "Time-dependent inactivation for identified (or “relevant” or specific “model”) viruses at the critical inactivation steps should be obtained as part of the process evaluation for these viruses. Purified bulk should be tested using suitable methods with high specificity and sensitivity for detecting the virus in question. For the purpose of marketing authorisation, data from at least 3 lots of purified bulk manufactured at pilot plant scale or commercial scale should be provided." for clarity as this is already mentioned before the description of the individual classes and this applies to class A and B as well.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
PTC Therapeutics	367	369	5	The guidance should mention that AAV would not be considered an adventitious "virus-like particle" in unprocessed bulk, if it's the API. The guidance should also mention “control cell testing”, in addition to "unprocessed bulk" because of this reason. The guidance does mention testing control cells in footnote ‘h’ of table A-5, but it could be good to call out in the main body too.	
EFPIA	370	372	5	"model" virus: example can be given and C-type particle are the important ones	MoMuLV would be a model for endogenous rodent simple C-type viruses.
ProPharma Group <Erik Schagen & Kristiena Abbink>	370	382	5	In Case B it is indicated that for rodent cell lines at least 3 lots of purified bulk need to be tested and results provided for a marketing authorisaton. This is not aligned with Table 4, "Test for virus in purified bulk" for Case B.	Align the description under Case B with the action required in Table 4.
Alliance for Regenerative Medicine	370	374	5	Case B: In rodent cell lines, if only a rodent retrovirus (or a retrovirus-like particle that is believed to be non-pathogenic, such as rodent A- and R-type particles) is present, the process evaluation using a specific “model” virus (such as a murine leukemia virus) should be performed. Purified bulk should be tested using suitable methods with high specificity and sensitivity for the detection of the virus in question.	Text is related to Case B in Table 4. Further text (Lines 375-382) says for certain well characterized cell lines/retrovirus-like particles, testing of purified bulk is usually not recommended. In Table 4 (after line 1028), the Case B Action Plan for Status “Test for virus in purified bulk” is simply “no”. The Case B text (Lines 370-382) and Table 4 may not be perfectly aligned. Proposed change: Table 4 Case B Action Plan for Status “Test for virus in purified bulk” should be “yes/no”, with a footnote indicating the Status may be dependent on the cell line and directing the reader back to the Case B text in lines 370 – 382.
Charles River Laboratories	372	375	5	This is in the opposite to table 4 which defines no testing on purified bulk. I assume Case B in table 4 refers to the exceptions (CHO, C127, BHK, (Sf9))as outlined in line 375 to 382 but this should clearly be indicated in table 4	Differentiate two case B scenarios in table 4 or adding another footnote
EFPIA	372	373	4	Put "purified bulk" definition in glossary	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
PPTA	372	377	5	“Purified bulk should be tested using suitable methods with high specificity and sensitivity for the detection of the virus in question. For marketing authorisation, data from at least 3 lots of purified bulk at pilot plant scale or commercial scale should be provided. Cell lines such as Chinese Hamster Ovary (CHO), C127, BHK and murine hybridoma cell lines have frequently been used as substrates for drug production with no reported safety problems related to viral contamination of the products.” This is in contradiction to Table 4 (lines 1027-1028), where no tests on purified bulk are required for Case B cells.	To align with requirements in Table 4: Replacement of term “purified bulk” by “unprocessed bulk”: "Purified unprocessed bulk should be tested using suitable methods with high specificity and sensitivity for the detection of the virus in question. For marketing authorisation, data from at least 3 lots of purified bulk at pilot plant scale or commercial scale should be provided. Cell lines such as Chinese Hamster Ovary (CHO), C127, BHK and murine hybridoma cell lines have frequently been used as substrates for drug production with no reported safety problems related to viral contamination of the products."
EFPIA	375	377	Section 5	Leverage sentence "Cell lines such as Chinese Hamster Ovary (CHO), C127, BHK and murine hybridoma cell lines have frequently been used as substrates for drug production with no reported safety problems related to viral contamination of the products. " to explain what "well-characterized" means. For example, the term is not used in this part of the document but instead it's buried as a footnote in Table 1, line 1000. It would be good to have this correlation of "well-characterized" and "CHO" in the main text, considering the prevalence of this cell line in the production of biologics.	See column F
EFPIA	379	380	5, Case B	Please add NS0 cell line because that is also widely used with no reported safety problems.	See column F
EFPIA	382	382	Section 5	Major consensus topic: Document structure & consistency Consider replacing the word "extensively" with "well" characterised, for consistency.	Consider replacing the word "extensively" with "well" characterised, for consistency.
PPTA	384	384	5	(e.g, Sf9 rhabdovirus (such as….)	to clarify
EFPIA	388	390	5	Add “if applicable” because non-enveloped viruses might not be inactivated under process conditions without harming the product.	Add “if applicable”

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Albrecht Gröner	390	391	5	Testing the purified bulk using suitable methods with high specificity and sensitivity for detecting the virus in question. It should be considered that testing - with an inherent limit of detection - is less sensitive than comparing the virus load in the unprocessed bulk and the virus reduction capacity resulting in an appropriate margin of virus safety	Testing of purified bulk should be replaced by a safety margin (i.e, according to Annex 5). The safety margin should be defined, e.g., < 10-3 virus particles/dose. Furthermore, in ANNEX III: CPMP/117/95 of CPMP/BWP/269/95 rev. 3 it is stated that - despite uncertainties at that time regarding the validity and sensitivity, e.g., missing International Standards for HCV RNA - plasma pool testing for absence of HCV RNA should be performed (in contrast to the requirement by the FDA to test the final product by PCR) [Compare embedded Excel file Virus Safety of Purified Bulk]
EFPIA	393	394	5		Correct the beginning of sentence to “If a known virus infectious to humans is identified…”
PPTA	393	393	5	Unclear if Case D refers to material that is known to have a human infectious virus present, or material that has the potential to contain a human infectious virus.	Proposed revision: "If a known virus is infectious….and remove the "." after footnote 1) .
EFPIA	395	397	5	Add “if applicable” because non-enveloped viruses might not be inactivated under process conditions without harming the product.	Add “if applicable”
Asahi Kasei Bioprocess Europe S.A./N.V.	398	399	5	The sentence "The process should be shown to remove and inactivate the selected viruses during the purification and inactivation processes" should be modified as virus filtration applied for virus removal is not a purification process step but a dedicated, orthogonal virus removal step	The process should be shown to remove and inactivate the selected viruses during the down-stream processes with inherent inactivation, dedicated virus removal, and purification steps
Albrecht Gröner	398	399	5	compare comment line 17	The process should be shown to remove and inactivate the selected viruses during the down-stream manufacturing process steps with integrated inactivation, dedicated virus removal, and purification steps
EFPIA	399	401	5	Add “if applicable” because non-enveloped viruses might not be inactivated under process conditions without harming the product.	Add “if applicable”
Charles River Laboratories	409	411	5	Required measures for such cases are not described different to the other cases (case C and D specifically). Footnote 9 of table 4 provides more detailed info e.g. that each purified bulk (not only three lots) should be tested for the helper virus using either infectivity assays or alternative methods; also viral load determination is requested.	Case F should be supplemented by the content of footnote 9 of table 4 and similar information given like for case C and B

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
ProPharma Group <Erik Schagen & Kristiena Abbink>	409	411	5	In the description of Case F nothing is mentioned about the required testing of the purified bulk. From footnote 9 under Table 4 it is understood that absence of the residual helper virus should be confirmed for each purified bulk.	It is suggested to include the requirement of testing each purified bulk in the description under Case F as well.
Parexel International	415	418	6	Consider removing the passage beginning 'Past instances..' up to '…reinforces that' as this text is not technical guidance but commentary.	Consider removing the passage beginning 'Past instances..' up to '…reinforces that' .
Alliance for Regenerative Medicine	416	450	Section 6 through to (including) Section 6.1	This section seems to be a good lead-in to the previous section (Section 5: Rationale and Action Plan for Viral Clearance Studies and Virus Tests on Purified Bulk)	Background information in Section 6 (e.g., definitions of "relevant" and "model" viruses) is highly applicable to Section 5. Consider moving Section 5 into, or after, Section 6.
EFPIA	417	419	6	Based on this reasoning, risk assessment should be adequate for products from fully characterized cell lines, such CHO, rather than experimental viral clearance studies.	See column F
Charles River Laboratories	419	420	6		Suggest to replace "….in a well documented and controlled manner" by "…under a quality assuarnce system" (GMP, GLP, ISO,…)
PTC Therapeutics	421	423	6	It seems that the viral clearance rationale appears in 2 places (lines 439-441 and lines 421-423), but the messaging is slightly different. Perhaps, these rationales could be combined together and more details provided at the beginning of this section (section 6)	
Charles River Laboratories	423	425	6	The spiking (see also line 527) "a virus" implies only one virus is being spiked. Are there any considerations to allow for a multi virus spike given that appropriate controls are in place (specificity of the quantification assay) and clearance is not impact compared to individual spikes	Suggest to indicate multi spike option when well controlled
Charles River Laboratories	430	432	6	Unclear - is this required for endogenous viruses only or should it be better clarified based on the different cases (A-F)	Suggest to replace "…the amount of endogenous virus particles….." by "….the amount of viruses detected in bulk harvest (Case B, C, D, E, and F; see table 4)….."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	430	432	6	Major consensus topic: Virus clearance study design Original text: “In general, in order to determine the amount of endogenous virus particles that enter the purification process, quantification should be performed on three cell cultures campaigns, lots or batches.” There are situations when three culture campaigns, lots, or batches are not needed (such as Case A).	Amgen recommends the following revision: “In general When appropriate, in order to determine the amount of endogenous virus particles that enter the purification process, quantification should be performed on three cell cultures campaigns, lots or batches.”
EFPIA	430	432	6	Major consensus topics: Virus clearance study design / Document structure & consistency If indeed lot and batch used interchangeably as defined in ICH Q7, better use the notation “batch (or lot)”. How is campaign defined? A series of sequential runs within a production slot? If quantification should be performed on 3 campaigns, should it be done for each run within a campaign or a selected run in each campaign?	for consistency reasons, EFPIA suggests to use the notation “batch (or lot)” throughout the document, and avoid the term "campaign"
Parexel International	431	431	6	three cell cultures campaigns' should be corrected to 'three cell culture campaigns'	three cell cultures campaigns' should be corrected to 'three cell culture campaigns'
ViruSure GmbH- Andy Bailey	436	437	6	Study design should include controls to determine to what extent virus is being cleared by inactivation, and what contribution is coming from removal	Suggest text: "For each production step assessed, the possible mechanism of loss of viral infectivity should be described with regard to whether it results from inactivation or removal, or a combination of both."
Charles River Laboratories	436	437			"……..results from inactivation or removal or a combined effect.
EFPIA	439	441	6	Major consensus topic: Virus clearance study design It is stated that "Viral clearance evaluation studies are performed to 1) demonstrate the clearance of a virus known to be present in the MCB In agreement with the new scope of the guideline, viruses known to be present may not only come from MCB, but may come from the type of expression system used.	Proposition : "Viral clearance evaluation studies are performed to 1) demonstrate the clearance of a virus known to be present endogeneously in the cell substrate, or brought by the expression system (e.g. helper virus)
PTC Therapeutics	439	441	6	It seems that the viral clearance rationale appears in 2 places (lines 439-441 and lines 421-423), but the messaging is slightly different. Perhaps, these rationales could be combined together and more details provided at the beginning of this section (section 6)	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	443	443	6	"mathematically" is not a clear goal. A better goal is to avoid infection. For example, typically 100 virus particles/dose is needed to initiate an infection, so it is not a safety concern if there is only one particle in one dose. This is used to manage parvo B19 situation for plasma thereapeutic proteins. For recombinant proteins, which has much lower risk, we require 4 logs of safety factor, requiring < 0.0001 particles per dose. This standard is extremely high with respect to real world risk, and leads to a waste of large amount of resouces.	See column F
EFPIA	445	448	6	Major consensus topic: Virus clearance study design It is stated: "The purpose of studies using viruses with a range of unknown or unexpected biochemical and biophysical properties is to characterise the robustness of the procedure rather than to achieve a specific inactivation or removal goal." Studies are done using viruses of known biochemical and biophysical properties. What is unknown is the properties of potential viral contaminants.	Proposition: "The purpose of studies using viruses with diverse biochemical and biophysical properties is to characterise the robustness of the procedure to clear viruses that are not known or expected to be present, rather than to achieve a specific inactivation or removal goal."
Octapharma Biopharmaceuticals GmbH	445	448	6	Priorization unclear with reagrds to aspects described in Section 5 and lines 650-651. Please clarify.	
Charles River Laboratories	450	450	6	"Therefore, achieving a specific clearance value is not needed": In other chapters validation of two steps minimaly is recommended and should be mentioned here. A note about minimal reduction of non specific model viruses would be helpful. E.g overall reduction of ≥ 4 log10 or the two steps should demonstrate reduction factors of >> 1 log10 (or >2 log10) each minimally	Suggestion:Two steps differing in the mode of virus clearance should be analyzed minimally, if possible. Reduction factors of ≥ 2log10 of each step or overall reduction of ≥ 4log10 are recommended minimally but acceptance will depend on risk assesment and case specific conditions. Prior knowledge application can further support.
Alliance for Regenerative Medicine	450	450	6	Therefore, achieving a specific clearance value is not needed.	Text refers to adventitious viruses. Add similar explanatory text (or direct reader to a different site in the document) re: endogenous viruses/virus-like particles.
Alliance for Regenerative Medicine	457	457	6.1.1	not sure why quotation marks are needed all through the documents when referring to "model" or "relevant" viruses	define what a "relevant-" and "model-" virus is once and then remove the quotation marks elsewhere
Parexel International	465	466	6.1.1	some firms will validate filtration with PPV vs. MMV. These studies are probbaly just as valid, but MMV is the "relevant" virus. Make clear that highly related viruses (e.g. have same size & family) can also be used.	"highly related viruses (e.g. have same size & family; PPV and MMV) can also be considered "relevant" with appropriate justification
EFPIA	474	476	6.1.1.	Major consensus topic: Document stucure & consistency Change from “murine origin” to “rodent origin” because for example also applicable for CHO cells.	This can be accomplished by using a murine leukemia virus--a specific “model” virus in the case of cells of rodent origin.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	477	478	6.1.1	Can agency clarify validation expectations to use endogenous virus particles in viral clearance experiemnts and provide an example how RVLPs clearance data will be used in overall clearance strategy?	EFPIA consensus Jan 27, 2023: insert “for example retrovirus-like particles (RVLP)” in line 478, and add RVLP as an option for virus clearance studies in footnote to Table 4
Parexel International	477	478	6.1.1	RVLP tracking is more likley to be used for actual in process screening at large scale (initial steps in process) vs. small scale studies. Make sure that it is clear that this is acceptable.	RVLP tracking as part of actual in process testing at large scale (initial steps in process) is an acceptable substitute of small scale studies of initial steps such as capture chromatography
Alliance for Regenerative Medicine	477	479	6.1.1.	For CHO cell-derived products, CHO-derived endogenous virus particles can also be used for viral clearance experiments. There is no infectivity assay for these particles, and the detection assay (e.g., molecular or biochemical) should be qualified for its use.	For clarity, it would be nice to add that a specific “model” virus (e.g., XMuLV) could also be used.
Asahi Kasei Bioprocess Europe S.A./N.V.	485	485	6.1.1	The use of “Robustness” here and the definition of “Process Robustness of Viral Clearance” in the glossary are confusing, since the latter is defined in this document as having two different, distinct meanings. This ambiguity has previously led to confusion in the industry and should be rectified in this document. ICH Q8 already defines “Process Robustness” as “Ability of a process to tolerate variability of materials and changes of the process and equipment without negative impact on quality.”	We recommend to provide two definitions: “Process Robustness” as “Ability of a process to tolerate variability of materials and changes of the process and equipment without negative impact on quality” in alignment with ICH Q8, and povide an additional term “Viral Clearance Robustness” as “Ability to clear a wide range of specific and non-specific model viruses”. Also, through the text, we recommend to use these terms strictly rather than the ambiguous term "robustness".
Alliance for Regenerative Medicine	488	490	6.1.1.	Preference should be given to viruses that display a significant resistance to physical and/or chemical treatments.	This is in relation to a general characterization of the process to remove/inactivate viruses. The indicated text could be taken to mean that, given sufficient inactivation, demonstration of further clearance of enveloped viruses by orthogonal methods isn’t necessary. Additional clarity around requirements clearance by orthogonal methods for both enveloped and non-enveloped viruses would be helpful. May be covered in Section 6.2.3, line 522. Definitely covered in Section .3, lines 647-649
EFPIA	491	493	6.1.1	Major consensus topic: Document structure and consistency Include reference to Annex 2	suggest to include reference to Annex 2
PPTA	509	510	6.2.1	No mention of GLP requirements for laboratories which conduct viral validation studies	Therefore, viral clearance studies should be conducted in a separate laboratory (GLP certified according to OECD principles)…

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Parexel International	510	511	6.2.1	In many cases viral clearance studies are performed at CTO sites. "In conjunction with production personnel" implies that manufacturing personnel must go to a CTO site- probably a good idea, but can this be stated	"production personnel with experience in the specific process"
Asahi Kasei Bioprocess Europe S.A./N.V.	513	520	6.2.2	Many viral clearance studies are performed using worse-case conditions, which may be outside of the intended manufacturing process ranges.	It should be clarified that runs may be performed at worse-case conditions that may be outside the acceptable range of the manufacturing process.
EFPIA	514	514	6.2.2	Major consensus topic: Virus clearance study design It is stated "The validity of scaling down should be demonstrated.". It can be understood as a full validation package where as the objective is to demonstrated the representativeness	EFPIA consensus Jan 26, 2023: Replace first two sentences of this paragraph by "The representativeness of scaling down should be demonstrated." In addition insert “For example” in the sentence listing specific process parameters in lines 515-518.
Alliance for Regenerative Medicine	514	515	6.2.2.	States "The level of purification of the scaled down version should represent the production procedure as closely as possible."	Delete "level of purification of the"
Parexel International	517	517	6.2.2	Consider using the word 'resin' instead of or in addition to the word 'gel'	Consider using the word 'resin' instead of or in addition to the word 'gel'
Albrecht Gröner	518	518	6.2.2	for clarification	conductivity should be added after salt
Pall Life Sciences	519	519	6.2.2	A similar elution profile should result. Only applicable for binding.	"A similar chromatographic profile should result…'
Lonza	519	519	6.2.2	Change wording for 'A similar elution profile should result…' elution is typically only for binding, what needs to be achieved is for the whole chromatographic profile (binding and flow through)	A similar chromatographic profile should result…' rather than restricting or limiting the evaluation to the elution phase.
BioPhorum	519	519	6.2.2	Change wording for 'A similar elution profile should result…' elution is typically only for binding, what needs to be achieved is for the whole chromatographic profile (binding and flow through)	A similar chromatographic profile should result…'
Parexel International	519	519	6.2.2	"similar elution profile" is a good start, but similar step yield is also important (large vs. small scale).	"and step yield"

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Parexel International	524	525	6.2.3	"exact definition of an individual step should be considered" is useful- but it isn't clear how pre-filters should be treated in a viral clearance study. Should they be part of the small scale model, or should spiking take place after pre-filtration. The second option make filtration studies difficult in that clogging occurs early.	The exact definition of the individual step should be justified, for example approach of validating pre-filter plus virus filter as one unit operation.
Parexel International	530	530	6.2.3	"virus load in the different fractions be investigated"- does this need to be performed by very well characterized removal steps like filters and many columns. Where the viruses are partitioned has already been established scientifically.	Remove requirement
Charles River Laboratories	531	533	6.2.3	This sentence is not clear. Using less virucidal buffers would have an impact on the down scale protocol and might be considered no more representative (e.g. in chromatography steps). Or is the meaning to analyze the inactivation capacity of the buffer itself in a seperate spiking experiment to differentiate the viral clearance of a step coming a: from removal (e.g. a chromatography step, filtration step,...) and b: from the buffer?	
Parexel International	536	537	6.2.3	"Quantitative assays not associated with infectivity may be used if justified." really isn't strong enough. Q-PCR is used in many viral clearance studies- and a strong statement that these assays are fine for measuring virus removal should be made.	Q-PCR is used in many viral clearance studies for steps that remove viruses (e.g. chromatography)- Q-PCR is an example of a justifiable quantitative assays not associated with infectivity
EFPIA	543	543	6.2.4	Major consensus topic: Evaluation of virus clearance studies Major Propose to be inclusive of inactivation and removal and open possibility to claim both mechanisms in same step.	Proposal to change to: "....as related to inactivation and/or removal."
EFPIA	543	545	6.2.4	Major consensus topic: Evaluation & characterisation of virus clearance studies The sentence here is repeatly redundant comparing to line 676-680.	suggest deleting the sentence of "If little clearance of infectivity is achieved by the production process and the clearance of virus is considered to be a major factor in the safety of the product, specific or additional inactivation/removal steps should be introduced".
Octapharma Biopharmaceuticals GmbH	546	550	6.2.4.	Example of segregation of inactivation and removal by two different chromatorgraphies is misleading.	Explicitly state how often similar modes of inactivation may be stated/accounted in the overall process.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Charles River Laboratories	557	558	6.2.5	Describing an inactivation curve by just one intermediate sample (next to the "0" sample and the minimal exposure time samples) is scientifically questionable. It depends on the time frame and intermediate samples should be selected reasonably. E.g. if fast inactivation is expected early intermediate samples should be collected while a slow 2 phase inactivation curve would require intermediate sampling closer to the end of the minimal exposure time.	Suggestion: Intermediate samples should be resonable selected based on the inactivation characteristics like expected speed of inactivation, duration of incubation, critiality of incubation variation, etc..
Pall Life Sciences	560	562	6.2.5	However, for inactivation studies in which non-specific “model” viruses are used or when specific “model” viruses are used as surrogates for virus particles such as the CHO intracytoplasmic retrovirus-like particles, reproducible clearance should be demonstrated in at least two independent studies. Can two indepdendent studies be defined (e.g. two different batches of product? Two separate spiking studies with same lot of product?)	
Octapharma Biopharmaceuticals GmbH	563	563	6.2.5	Clarify if "studies" is equivalent to "runs" / "Tests"	Define study, run and test under respective section.
Pall Life Sciences	566	568	6.2.5	When inactivation is too rapid to plot an inactivation curve using process conditions, appropriate controls should be performed to demonstrate that infectivity is indeed lost by inactivation. Can we add examples of appropriate controls?	
Lonza	566	568	6.2.5	Wording has not changed when compared to R1. However there is an opportunity with R2 to clarify what is meant by this sentence or to give examples, as this is not clear. Maybe an option would be to remove the sentence as controls within a VC study would normally cover this topic.	This is currently ambiguous and unchanged from version 1. In general VC studies contain controls within the assays that demonstrate inactivation as control samples so maybe an example of where this approach may not be appropriate or lacking in demonstration of a mode of inactivation by virtue of it being rapid.
BioPhorum	566	568	6.2.5	Wording has not changed when compared to R1, however there is an opportunity with R2 to ckarify what is meant by this sentence or to give examples, as this is not clear for industry. AN alternative would be to remove the sentence	
ViruSure GmbH- Andy Bailey	567	568	6.2.5	Some have advocated using conditions less harsh for inactvation (e.g. using a concentration of detergent significantly lower than that used in manufacturing). The relevance though of data generated under such unrealistic conditions has sometimes been questioned, so some guidance here would help	Some examples of what "appropriate controls" might be considered would be helpful

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Biosimilar Medicines Group - Medicines for Europe	570	580	6.2.6	Additional elaboration or examples of prior knowledge sufficient to support repeated resin use for other chromatography types other than the protein A affinity capture chromatography would be helpful. The adequate minimum number of in-house experiences to support the viral clearance capability of used resin and whether these experiences should be from at least 3 products or experience with a single product is sufficient could be clarified in the guideline. Also, the extent of the prior knowledge necessary to support repeated resin use could be further explained or examples could be provided. For example, if prior knowledge indicates that the viral clearance capabilities are comparable between a new and used resin (i.e. 150 cycles), would additional viral clearance data be necessary to extend the maximum number of resin cycles to 200 cycles or would it be not necessary as with the Protein A affinity capture chromatography?	
EFPIA	571	572	6.2.6	Major consensus topic: Evaluation & characterisation of virus clearance studies This is the first time to introduce critical process parameters so it is confusing under "Function and Regeneration of Columns" section. Suggest moving or adding critical process parameters discussion to section 6.2.2 Scaled-Down Production system since non-chromatography steps also need to define critical process parameters.	EFPIA consensus Jan 17, 2023: suggest to replace "critical process parameters" by " potential impact parameters" as in Annex 6
Alliance for Regenerative Medicine	571	573	6.2.6.	States "Chromatography media/resin lifetime use should be indicated, and critical process parameters that impact viral clearance should be defined." Under what circumstances should viral clearance studies be performed to support chromatography media/resin re-use?	Suggest to add e.g. "Viral clearance studies may be required to support media/resin re-use if a risk of detrimental impact to viral clearance and/or inactivation is indicated".
Biosimilar Medicines Group - Medicines for Europe	572	573	6.2.6	Suggestion to clarify how Applicants can define CPPs for virus removal for chromatography steps when it is impractical to conduct process characterisation studies that include virus spikes	Provide specific guidance on the justification of theoretical worst case conditions via risk assessment using prior knowledge and literature that can then be included in the clearance study
Rentschler Biopharma SE	574	580	6.2.6	We positively noticed that prior knowledge as in-house experience could be applied to substitute product-specific virus spiking studies with used (end-of-lifetime) chromatography media/resins. According to the guideline draft this applies to different chromatography types (e.g. anion / cation exchange). Nevertheless, we do miss a more detailed guidance towards limitations of using prior-knowledge data. Is only specific data from the identical resin rated as representative/equivalent to justify data based end-of-lifetime assessment? Or is also prior knowlege/inhouse experience with resins of comparable ligand properties and/or backbone structure legitimating to substitute product-specific virus spiking experiments? What are the relevant performance indicators (e.g. process-related impurity clearance, peak spreading, backpressure trend, ...) confirming performance consistency troughout resin lifetime to preclude the need for product-specific viral clearance studies?	Please include additional information defining in more detail equivalence of prior-knowledge / in-house experience ultimately justifying data-driven assessment.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Parexel International	579	579	6.2.6	"equivalent prior knowledge including in-house experience "- this will be useful for large firms with many other products- what about firms with only a few products- can they reference other prior knowedge like ASTM standards?	
Rentschler Biopharma SE	581	584	6.2.6	To prevent potential carry-over of any virus retained by the production system a demonstration of cleaning/regeneration effectiveness should be provided. In terms of chromatography this implies to include testing of virus carry-over within virus clearance studies by running a non virus-spiked run. No further general guidance reflecting the minimal authority expectation is given to enable the adequate design of such carry-over testings. Further explanation: For a variety of chromatographic method aproaches, in particular for those forcing process intensification (e.g continuous manufacturing), regeneration and cleaning procedures are not applied excessively and continuously between chromatography cycles to maximize resin lifetime. Hence, testing inter-cycle cleaning/regeneration effectiveness in virus clearance studies potentially leads to underestimation of cleaning/regeneration effectiveness. Furthermore, it assumes only a virus contamination event for a single cycle. However, the worst case of a potential virus contamination introduced into each cycle of one batch by intermediates or materials (e.g. buffer) is considered to be more realistic. Accordingly, inter-batch cleaning/regeneration should rather be tested for demonstration of effectiveness to exclude potential carry-over of viruses into the subsequent production batch.	Inclusion of additional guidance reflecting authority expectation (e.g. at least the inter-batch cleaning and regeneration effectiveness should be demonstrated).
Rentschler Biopharma SE	581	584	6.2.6	According to the guideline draft, data should be provided showing adequate virus removal or destruction for example by cleaning and regeneration procedures to allow for reusing the system. A more detailed definition of the term "adequate" would enable the filing party to meet authority expectations much better. In our view, one very useful option is the calculation of a carry-over treshold alterating the LRF of a process step determined within a virus clearance study at max in the range of the accepted assay variation of 0.5 log10. This treshold could be very easily calculated and could serve as a much better orientation. In additon, such a threshold would allow to appropriately judge the impact of a virus carry-over which might be different on effective vs. moderate virus removal steps.	More detailed definition of adequate virus removal/destruction by including the option to work with certain virus carry-over treshold calculations.
Parexel International	581	582	6.2.6	"any virus potentially retained by the production system would be adequately destroyed or removed before reusing the system". This is usually done by carry-over experiments. However, what is the value of these studies, in terms of actionable steps? If a small number of RVLP particles are carried forward in a protein A step- what is the follow up? Also, since the other spiking studies with other viruses are artificial anyway- what is the follow up if a small amout of a hardy virus like MMV is found in the bank run of the next cycle? It shouldn't be there normally anyway in a clean facility	Remove requirement

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	582	584	6.2.6	Major consensus topic: Function and regeneration of columns Original text: “For example, evidence may be provided demonstrating that the cleaning and regeneration procedures inactivate or remove virus.” We recommend allowing the use of prior knowledge as evidence.	EFPIA recommends the following revision: “For example, evidence, such as prior knowledge, may be provided demonstrating that the cleaning and regeneration procedures inactivate or remove virus.”
Asahi Kasei Bioprocess Europe S.A./N.V.	586	620	6.2.7	The quality and purity of the virus spike can produce non-representative artifacts in the process if they are not aligned with the purity of the representative feedstock. This is especially apparent when using low purity virus stocks for process which are downstream and have highly pure feeds.	We recommend to add a bullet point stating “Virus spike purity should be similar or more pure than the purity of the product; especially, highly pure stocks should be used to spike downstream steps to avoid introducing non-representative impurities that may impact process performance.”
EFPIA	587	620	6.2.7.	Major consensus topic: Evaluation and characterisation of virus clearance studies Add to address the risk that non-encapsulated viral genomes influence the virus clearance quantification when using molecular biology assays like e.g. qPCR results	Add new bullet: In case molecular biology assays are used for quantification, the effect from non-encapsulated viral genomes should be minimized.
Parexel International	587	587	6.2.7	"Care should be taken in preparing the high-titer virus to avoid aggregation" should also state- "and maximize purity (v.s. extraneous proteins)". The arguements that "crude preps" are "more realistic of a bioreactor crash" really aren't valid as the HCPs in the virus prep wil lbe from adherent, non-CHO host cells grown in tissue culture plates.	and maximize purity (v.s. extraneous proteins)
Charles River Laboratories	603	605	6.2.7	This sentence is not clear see suggestion. Also, toxicity testing is not an alternative to intereference testing (the sentence says "….toxicity or interference…."). Typically both testings are applied but intereference assay minimally	Suggestion: All process samples intended for virus quantification and differing in its composition (product concentration, buffer composition, pH, etc) should be evaluated for cytotoxicity and interference in virus quantification assay.
Octapharma Biopharmaceuticals GmbH	613	616	6.2.7	Section implies that usage of same or similar clearance may be accounted for as reduction step, despite same working principle.	Meant could be that a platform approach over different, independent production processes might not be applicable as the positioning in the respective manufacture might be contact

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Albrecht Gröner	613	616	6.2.7	This paragraph is not in line with the requirement for "implementing two distinct effective steps that complement each other in their mode of action is recommended" (line 648-649); furthermore, 6.2.4, line 547 - 550 requests to distinguish between removal and inactivation.	Many purification schemes use the same or similar buffers or columns repetitively. When the overall virus reduction factor for a complete production process is based on the sum of the reduction factors of such purification schemes, this approach has to be justified, e.g., the effectiveness of virus elimination by a particular process may vary with the stage in manufacture at which it is used and the presence of accompanying proteins and other impurities clearly affecting the virus reduction capacity.
EFPIA	621	621	6,3	Global comment: "virus cleraance" is used here but "viral clearance" elsewhere. Suggest to use "viral clearance" only because that was historically used. Using "virus clearance" may lead to missing information when searching relavent content.	Minor editorial comment
EFPIA	624	627	6.3	Include Case F? Provide some guidance on minimum log clearance expected for model viruses because “excess capacity” is rather vague?	
EFPIA	628	629	6,3	Major consensus topic: Document structure & consistency Major Consistency & scientific accuracy. Does not matter where testing is done as long as testing is done before the claimed purification proces. Replace "unprocessed bulk" by virus which may be entering the purification process as described in line 431	Replace "... virus which may be present in unprocessed bulk." by "... virus which may be entering the purification process."
EFPIA	630	631	6,3	Major consensus topic: Document structure & consistency Consistency & scientific accuracy. Does not matter where testing is done as long as testing is done before the claimed purification proces. Replace "unprocessed bulk" by virus which may be entering the purification process as described in line 431	Replace "... virus in the unprocessed bulk" by "... virus which may be entering the purification process."
BioPhorum	630	630	6.3	Calculation of estimated particles per dose. However, it is not possible to perform such a calculation for AAV, since the TEM testing that forms the start of the calculation is not possible for AAV (the product is a virus-like particle, therefore it is not possible to distinguish between the product and an endogenous virus-like particle in the product stream via transmission electron microscopy, so direct testing of the harvest bulk is not possible). What would be equivalent?	Propose that clarification is added to confirm that the estimation of particles per dose is not relevant for AAV, and that just providing reduction factors per step is sufficient to demonstrate viral clearance. Conversely, if the estimation is required, request that further information is provided on how this may be achieved (e.g. using TEM result from control culture).

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	646	647	6,3	Major consensus topic: Virus clearance study design It is stated "It is recommended to design a downstream process that clears a wide range of potential virus contaminants.", where as the process design also aims at clearing process related viral contaminants	Proposition: It is recommended to design a downstream process that clears a wide range of viruses (potential adventitious, endogenous and/or helper virus)."
Pall Life Sciences	647	649	6,3	In this context, whenever feasible and not adversely affecting the product, implementing two distinct effective steps that complement each other in their mode of action is recommended. Perhaps "a minimal of two distinct..."	In this context, whenever feasible and not adversely affecting the product, implementing a minimum of two distinct effective steps that complement each other in their mode of action is recommended.
PPTA	648	648	6,3	Suggest revision of 'two distinct steps' to 'at least two distinct steps'	fc
EFPIA	649	649	6,3	Major Adapt to current practice as in some cases it may not be possible to achieve 4 logs of clearance in one step.	Propose to replace "effective" by "provide reproducible reduction of non-enveloped virus."
Asahi Kasei Bioprocess Europe S.A./N.V.	650	651	6,3	An effective virus removal step generally gives reproducible reduction of virus load in the order of 4 logs or more shown by at least two independent studies.	Is the reduction of 4 logs or more a requirement? The description is unclear, since 450 states that ". Therefore, achieving a specific clearance value is not needed. ." Is there any contradiction with this statement?
Asahi Kasei Bioprocess Europe S.A./N.V.	650	651	6,3	An effective virus removal step generally gives reproducible reduction of virus load in the order of 4 logs or more shown by at least two independent studies.	Addition of the statement: "at least two independent studies" could include results from n = 2 (duplicate run) on the same test date or with the same lot of process solution. Reason: It should be specified whether "at least two independent studies" are possible with results from n = 2 (duplicate runs) on the same test date or with the same lot of process solution. Industry's opinion is that the execution of virus clearance tests is important and burdensome, and that n = 2 (duplicate run) is usually used. We would like to confirm that it is not mandatory to run the test on different test dates or with different lots of process solution.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Charles River Laboratories	650	653	6.3	A: The word "effective" is conected to a minimal reduction factor (4 log10) whereas in lines 637 to 645 effectiveness is stressed requiring different criteria and the log10 reduction is just one. B: it would help if log numbers can be stated a bit more specific. It's frequently point of discussions. If one wants to consider confidence limits as requested in Annex 3, chapter 4 (lines 1127-1131) one could be even more specific but it would require some more detailed description of statistics as requested in chapter 6.5.; maybe added to Annex 3.	A: Suggest to replace the term "effective" when correlated to a log10 reduction number by the terms "strong" or "significant". B: Instead of saying "….in the order of 4 logs or more….", "…….....≥ 4 logs10; same with subsequent log numbers: >1log10 to < 4 log10 (instead of 3 log)
EFPIA	650	651	6,3	In the sentence "An effective virus removal step generally gives reproducible reduction in the order of 4 logs or more shown by at least 2 independant studies": proposal to replace "virus removal" by "virus clearance" as the mechanism for viral reduction could be either removal or inactivation.	"An effective virus clearance step generally gives reproducible reduction in the order of 4 logs or mor shown by at least 2 independant studies".
Octapharma Biopharmaceuticals GmbH	650	651	6,3	Would an LRF of 3.5 log be considered in the "order of 4 log"? Would this be matter of a case-by-case evaluation?	
ViruSure GmbH- Andy Bailey	651	651	6	The term "independent studies" can be interpreted in different ways	The term "independent spiked runs" is suggested; This could include runs performed in parallel or runs performed on different days
ViruSure GmbH- Andy Bailey	651	653	6,3	In some studies, the ability to achieve a reduction factor of 4 logs is limited because of e.g. high cytotoxicity from the test material. Such steps may still be considered effective where appropriate controls have been included in the study to confirm that virus is removed to below the LOD of the assay via a robust mechanism. What is being discussed in this sentence though refers specifically to steps where e.g. virus clearance is not complete, but reproducibly yields a reduction factor greater than 1 log	It is suggested to include a discussion of the scenario where it is not possible to achieve a reduction factor of 4 logs even with an optimised experimental design, to differentiate from the scenario where virus reduction is reproducible but not complete and less than 4 logs
Pall Life Sciences	651	651	6,3	Need further definition of "two independent studies". Independency needs to be defined in the glossary, what is required to claim independency?	Add defintion to glossary
Pall Life Sciences	651	653	6,3	"However, it is recognised that steps giving a reproducible reduction in the order of 1 to 3 logs contribute towards viral safety and can be considered for assessment of overall virus reduction." This is a contradiction with lines 674 and 700.	" in the order of >1 to 3 logs

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Asahi Kasei Bioprocess Europe S.A./N.V.	651	653	6,3	However, it is recognised that steps giving a reproducible reduction in the order of 1 to 3 logs contribute towards viral safety and can be considered for assessment of overall virus reduction.	However, it is recognised that steps giving a reproducible reduction in the order of 1 to 3 logs contribute towards viral safety and can be acceptable in consideration (or included) for assessment of overall virus reduction. Reason: In the process of removing non-enveloped viruses, depending on the process, it may not be possible to achieve 4 log or more even with all possible optimization. For this reason, we would appreciate it if you could consider specifying that if the process is reproducible and achieves a reduction of 1 to 3 logs, the clearance value can be included in the non-envelope virus removal process.
Lonza	651	651	6.3	What does "two independent studies" mean? Independency needs to be defined in the glossary, what is required to claim independency? This is not clear . More precise words are required to alleviate ambiguity. In this instance maybe replace with "two independent spiking runs". Although twice doesn't confer consistency from a statistical perspective.	Simply state - capability should be demonstrated in at least two independent spiking runs… and not infer statistical significance to doing something twice, simply demonstrating it twice and potentially not under identical conditions but separated to create a kind of design space.
Lonza	651	653	6.3	"in the order of 1 to 3 logs" please replace with "in the order of ≥1.0 to 3.0 logs" (≥ symbol), because of the criticality of 1 log limit, leaving it as in revision 1 is creating unnecessary confusion. Virus assays generally claim ±1.00 log as significant therefore a value of 1.00 would be included therefore this document edit should take the opportunity to eliminate the ambiguity. See comment below of line 674.	Generally ensure the document is less ambiguous regarding 1 log. In general viral assays have a statistical significance attached to them where 1.0 log is significant (unless otherwise specified) and less that 1 log is not. So 1.00 would be included in a cumulative reduction but 0.99 would not. This is an opportunity to correct a current misalignment and inconsistency.
EFPIA	651	651	6,3	Major consensus topic: Virus clearance study design Major: replace " two independent studies"	EFPIA suggests to replace "two independent studies" by "two independent virus spiked experiments"
BioPhorum	651	651	6.3	What does "two independent studies" mean? Independency needs to be defined in the glossary, what is required to claim independency? This is not clear . More precise words are required to alleviate ambiguity. In this instance, industry proposes to replace with "two independent spiking runs",	
BioPhorum	651	653	6.3	"in the order of 1 to 3 logs" please replace with "in the order of >1.0 to 3.0 logs" (insert symbol and digit), because of the criticality of 1 log limit, leving it as is is creating unecessary confusions	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
PPTA	651	651	6,3	Suggest to revise the word "studies" with red word as reported in column G	"….by at least two independent runs or experiments"
Biosimilar Medicines Group - Medicines for Europe	651	651	6,3	"two independent studies"	Replace with "two independent spiking runs"
Albrecht Gröner	653	656	6,3	Solvent/Detergent treatment, treatment with detergent alone, or incubation at low pH, have been very successful in clearing a wide range of enveloped viruses whereas virus filtration (nanofiltration) removes viruses based on size exclusion.	Process steps dedicated to virus inactivation/removal such as Solvent/Detergent treatment, treatment with detergent alone, or incubation at low pH, have been very successful in clearing a wide range of enveloped viruses and virus filtration removes viruses based on their size (size exclusion mechanism).
Charles River Laboratories	655	655	6.3		"….incubation at low/high pH…."
BioPhorum	655	655	6.3	"incubation at low pH"to be replaced with "incubation at low pH/high pH"	
Asahi Kasei Bioprocess Europe S.A./N.V.	656	657	6,3	Due to it's use of the term "effective", the line “Using virus filters … is also an effective virus clearance step for the smaller parvovirus or polyomaviruses” seems to present a requirement that virus filtration should achieve 4 or more logs of clearance of parvoviruses or polyomaviruses, but this is not explicitly clear. Although, this degree of clearance is usually observed for virus filtration, there are many documented cases of challenging conditions and processes where lower clearance is observed. Historically, this has been acceptable as long as the assessment of overall virus reduction by the process provides sufficient virus clearance.	It should be clarified whether the intent of this document is to require that virus filtration should achieve 4 or more logs of clearance of parvoviruses or polyomaviruses, but this is not explicitly clear. If not, we recommend to reword this line or add terminology stating that reproducible reduction in the order of 1 to 3 logs can be acceptable considering the assessment of overall virus reduction.
PPTA	656	657	6.3	Suggest to revise the phrase	"…of small viruses is also an effective methodology for viral clearance of parvoviruses or polyomaviruses."
Albrecht Gröner	656	657	6,3	"… smaller parvovirus or polyomarivruses" seems inappropriate as the size of parvoviruses is comparable within the family (range approx. 17 to 24 nm) [TYPO: polyomaviruses]	Using virus filters designed for removal of small viruses is also an effective virus clearance step for the smaller viruses as parvoviruses or polyomaviruses
PPTA	657	657	6.3	Typo: *polyomarivruses*	polyomaviruses

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Alliance for Regenerative Medicine	657	657	6.3	correct spelling	write polyomaviruses not 'polyomarivruses'
BioPhorum	658	658	6.3	"Xenotropic Murine Leukemia Virus (XMuLV)" pto be replaced by "Murine Leukemia Virus (MuLV) as this covers the two types used: X-MuLV and Mo/A-MuLV. Both X-MuLV and Mo/A-MuLV are used in Viral clearance tests.	
EFPIA	661	672	6,3	Major consensus topic: Evaluation & characterisation of virus clearance studies Minor (clarification): The paragraph speaks to "acceptable overall clearance" via removal and/or inactivation. Could we align terminology with other sections of the document by replacing 'separation' with 'removal' in this paragraph Line 669 speaks to "effective removal". could we replace this with "effective clearance", as the sentence refers to both separation and inactivation steps	Suggest to replace with "separation" with "removal" in the paragraph, and to replace "removal" in line 669 with "clearance"
EFPIA	661	663	6.3	Change to "multiple complementary (or different) inactivation steps" to indicate that in case of multiple inactivation step different inactivation modes are preferred?	
PPTA	661	663	6,3	Needs to be clearer on orthogonal steps	"…..multiple complementary (orthogonal) steps…."
Alliance for Regenerative Medicine	661	663	6.3	Acceptable overall clearance can be achieved by any of the following steps: multiple inactivation steps, multiple complementary separation steps, or combinations of inactivation and separation steps.	Add "complimentary" before "inactivation" in line 661, if appropriate.
Parexel International	662	662	6,3	re: multiple complementary separation steps", Some have argued that there should be at least one inactivation step in a bioprocess. This is based on experience form the plasma products industry- and low pH is pretty universal. While this is probably not abosolutely needed for viral safety, the document should encourage frims to have one.	"it would be desriable but not required to have at least one inactivation step in a bioprocess."
Charles River Laboratories	665	666	6.3		Suggest to replace: "........"model" viruses can be separated in a different manner than a target virus." by "….similar model viruses can behave differently."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
PPTA	665	665	6.3	Suggest to add "their"	"…membranes) and their precipitation properties,…."
PPTA	666	666	6.3	target virus	Replace with "relevant virus" to align with the rest in the text
EFPIA	667	668		Meaning glycosylation of viruses?	EFPIA would like to ask for clarification or deletion of this sentence
Parexel International	668	668	6,3	"surface properties such as glycosylation"- add charge differences- more likely to impact chromatographic behaviour.	"and charge differences"
Lonza	674	675	6.3	Line 652 "reduction in the order of 1 to 3 logs contribute..." In 674/675 "However, reduction in virus titre of the order of 1 log10 or less would be considered negligible and could be ignored unless justified" Wording is too vague; proposal is to replace by less than 1.0, or to address clearly the uncertainty linked to the result: such as statistic considerations like confidence limits? Current description can be interpreted multiple ways.	Modify wording to state ≥1 log would be considered significant or contributes to overall reduction but less than 1 log would not. No need to have the additional wording here of '1 log or less' just simply state of 1 log or more (as in above comment) contributes.
Charles River Laboratories	674	674	6.3		≤ 1 log10
BioPhorum	674	675	6.3	Line 652 "reduction in the order of 1 to 3 logs contribute" In 674/675 "However, reduction in virus titer of the order of 1 log10 or less would be considered negligible and could be ignored unless justified" Wording is too vague; proposal from industry to replace by less than 1.0, or to address clearly the uncertainty linked to the result: such as statistic considerations like confidence limits? The whole discussion needs to be more precise and consider values and confidence limits. Current description can be interpreted multiple ways.	
Parexel International	675	675	6,3	Add a line that the overall reduction factor should be calculated using a worst-case approach and therefore using the lowest values from the independent runs for each step.	The overall reduction factor should be calculated using a worst-case approach and therefore using the lowest values from the independent runs for each step

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
PPTA	690	692	6.4	Sentence difficult to read: "*For example, this could include if virus particles used for spiking and native virus from a respective production intermediate differ in purity or degree of aggregation* ;", consider omitting 'this could include…'	Proposal to change the wording to "*For example, this could include if virus particles used for spiking…* "
Pall Life Sciences	694	695	6,4	It is possible that virus escaping a first inactivation step may be more resistant to subsequent steps. Replace word "escape" with "evading"	It is possible that virus evading a first inactivation step may be more resistant to subsequent steps.
Lonza	700	701		1 log10 is correct here so the above comment on line 674 should align to this. So 1.00 log is significant and contributes statistically and 0.99 would not.	See the two comments above on consistency and the inclusion of 1 log. After all, 1 log means a change in titre of 10 fold !
Parexel International	703	705	6,4	"Furthermore, if reduction values achieved by repetition of identical or near identical procedures are included, they should be justified"- be a little careful here. What is to stop someone from running four virus filters in series and then claiming that the LRVs can be added togeather, giving a 20 log10 clearance factor. This arguement would be bogus, but this language doesn't prevent it.	
PPTA	703	705	6,4	Hard to see justification for aggregating data from repeat identical steps	
EFPIA	710	710	6.4.	sentence appears incomplete	EFPIA suggests to complete the sentence.
Charles River Laboratories	711	711	6.4		"Pilot-plant scale" is a bit misleading; we suggest "laboratory scale for viral clearance studies may differ ….."
EFPIA	711	712	6,4	Not sure why this is important as the scale-down is compared agains commercial scale.	Propose to remove bullet point.
PPTA	711	712	6,4	"Pilot-plant scale processing may differ from commercial-scale processing despite care taken to design the scaled-down process"; the requirement is unclear. Reference to pilot scale processing suggests larger volumes than spike studies, which would generally be lab-scale. Please calrify.	It is assumed that the following requirement is intended here: "Pilot-plant scale processing may differ from commercial-scale processing, which should be taken in consideration for design of the scaled-down process". Or if more appropriate, consider a change from "Pilot-scale" to "Bench-scale processing……".

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Alliance for Regenerative Medicine	711	712	6,4	The problem being pointed out in 711-712 is unclear. Is this referring to a concern with using pilot scale materials as test articles? Or is it stating that the scaled down clearance study may differ from commercial scale processing?	Advise to revise wording to clearly discriminate between the use of pilot scale manufacture to produce test samples of product and the scaled down model used to study viral clearance.
EFPIA	729	729	7	Major consensus topic: Prior knowledge/Protein-virus interaction major comment: There is no simple way to show that virus and product do not interact. Rational for proposed change: virus-product interactions which negatively affect virus clearance are an exception to the rule based on current understanding, important to reflect this in the guideline. For example, inactivation and virus filtration conditions are chosen so that we operate on a plateau regarding virus clearance capacity, independent from virus -product interactions	Recommend to use wording from the 2008 EMA virus safety for IMPs to meet the intent of this requirement: “If data for more than one product is available for the specific step, the effectiveness of virus reduction should be comparable in each case.”
Alliance for Regenerative Medicine	745	749	6.6.	Given that comparability has a specific meaning with respect to biological products and therefore this terminology here might be confusing - suggest instead to use e.g. similarity.	Instead of "comparability" suggest to use e.g. "similarity".
EFPIA	747	748	6,6	Major: In sentence "demonstration of comparability of the processes across manufacture of different products involved, comparability of the product intermediates, and an assurance that product-specific", the term Comparability as defined in ICH Q5E, section 1.4, sets a high bar of investigation including cell based assays and up to clinical trials more applicable to Drug Substance than process intermediates.	Change "comparability" to "Similarity" or"representativeness"
Albrecht Gröner	755	756	6,6	Clarification that platform technology / prior knowledge has to be based on very robust data covering variable composition of the process step (including upstream process steps) as well as properties of the virus studied is wellcomed. Therefore, the statement "If the data package does not sufficiently support the use of prior knowledge, product-specific viral clearance studies should be performed" is supported	
ProPharma Group <Erik Schagen & Kristiena Abbink>	757	759	6,6	"LRV claim" is left unexplained.	Include meaning of abbreviation LRV.
PPTA	757	757	6,6	Specify acronym of LRV, as the term “LRV” is mentioned for the 1st time-without explaining this abbreviation.	Include term (full length + Abbreviation) in the Glossary (Section 9) (Log reduction value, LRV)

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Albrecht Gröner	757	758	6,6	Abbreviation LRV is not disclosed (also not in Glossary)	When deriving a LRV (Log Reduction Value - Virus reduction factor in log10) claim using prior knowledge, the claim should be justified considering all LRVs from the relevant platform data. A conservative LRV claim is advised to avoid a risk for overestimating the reduction capacity of the process step. Otherwise, LRV may be covered in Glossary
EFPIA	760	760	7	Major consensus topic: Continuous manufacturing Recommend it be noted that these are examples - there could be different cases for which this is justified.	Change "cases" to "examples"
Alliance for Regenerative Medicine	763	773	Section 6.7.	Examples of changes (e.g., Major/Minor) via an Appendix that would stratify the necessity of re-evaluation of viral clearance. In addition, the implementation of prior knowledge may mitigate the risk of minor process changes.	
EFPIA	776	779	7	Major consensus topic: Continuous manufacturing The introduction is to broad and inconsistent with ICHQ13 continuous manufacturing.	Please introduce continuous manufacturing with ICHQ13 phrasing line 17-18 : Replace first sentence, line 776-779 by "CM involves the continuous feeding of input materials into, the transformation of in-process materials within, and the concomitant removal of output materials from a manufacturing process. "
EFPIA	782	782	7.	Reference is made to ICH Q13, which is currently a draft document. Q13 was released for public consultation on 27 July 2021.	It should be verified that the reference is valid.
EFPIA	790	793	7	Major consensus topic: Continuous manufacturing Minor: The statement provided is long and difficult to read which may limit comprehension. For example, the physical and chemical conditions to inactivate or remove viruses derived from experience or prior knowledge of batch production are applicable when the target state of control regarding process parameters, which are relevant for virus clearance is ensured even in dynamic processes (see Section 6.6).	EFPIA consensus Jan 24, 2023: suggest to re-phrase lines 790-793 to: "For example, the physical and chemical conditions to inactivate or remove viruses derived from experience or prior knowledge of batch production may also be applicable to continuous manufacturing processes."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	793	793	7	Major consensus topic: Continuous manufacturing Major: in the sentence " which are relevant for virus clearance is ensured even in dynamic processes (see Section 6.6).", the term dynamic process is not consistent with ICHQ13 and overlaps with batch process	Please delete dynamic and replace with CM process. Proposal: "which are relevant for virus clearance is ensured even in CM processes (see Section 6.6).
Pall Life Sciences	805	807	7,2	"The manufacturing process may be partially run in continuous or connected mode of operation and it is possible to use knowledge/experiences of virus clearance study design based on batch processes for the evaluation of unit operation if suitable". Suggest including an example of where knowledge of a virus clearance step from a batch could be applied to continuous manufacturing. While the principles of the virus clearance or inactivation step may be th same, in our experience, it takes a different startegy to apply this to a continuous process.	
EFPIA	805	806	7,2	Major consensus topic: Continuous manufacturing Minor: Should the definition/distinction of connected vs continuous unit operations be provided to clarify whether the concepts of CM are being applied there. See lines 841, 845, Q13 defines units operations as integrated which may be different than just connected.	EFPIA consensus Jan 24, 2023: suggest to re-phrase line 805: "...may be run in continuous or partially continuous (connected) mode…"
BioPhorum	805	807	7.2	"The manufacturing process may be partially run in continuous or connected mode of operation and it is possible to use knowledge/experiences of virus clearance study design based on batch processes for the evaluation of unit operation if suitable". Suggest including an example of where knowledge of a virus clearance step from a batch process could be applied to continuous manufacturing.	
Alliance for Regenerative Medicine	805	806	Section 7.2.	Utilization of scientific and prior knowledge and relevant experiences are all elements of designing suitable manufacturing process.	change "use knowledge/experiences" to "use scientific and prior knowledge"

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	812	813	7	The section omits a discussion of sampling strategy in continuous manufacturing other than at lines 812-813. We note that there is no discrete “unprocessed bulk” harvest pool sample in a continuous harvest mode, and therefore it is not clear if sampling should be at end of production or at more frequent intervals, based on risk considerations.	We suggest adding text providing context on sampling strategy for various continuous manufacturing paradigms. For example: “Continuous manufacturing can be implemented with a series of intermediate sub-batches that can be sampled as discrete homogeneous unprocessed bulk harvest pools. Alternatively, continuous manufacturing can be implemented in a continuous harvest mode with no intermediate pools prior to purification. In the latter case sampling of unprocessed bulk for viral contaminants could be, minimally, from the flow stream or surge vessel at the end of production. Sampling of flow stream/surge vessel at defined intervals prior to the end of production could also be considered as per a risk assessment.”
EFPIA	812	817	7.2.	Major consensus topic: Continuous manufacturing Section deals with "inadvertant disturbance" and virus contamination. In our view, the consequences are miles apart: In case of (confirmed) virus contamination the cultivation should be shut down. A virus contamination is not reversible. Other disturbances (e.g. low product concentraion due to insufficient aeration) may be remedied by diversion to waste until cultivation in "back to normal", after which production may be resumed (pending risk evaluation of incident). Continuous cultivations may run for months on end. It is desirable to be able to release materials concomitantly, and this may be achived when virus testing covering the harvest period has been performed with a compliant result. Harvest materials from the period in which the virus contamination occurred In addition, for viral clearance and inactivation, the expectations for addressing product diversion systems for CM in a event of a disturbance are not clear. Recommend to cross-reference ICHQ13 accordingly for simplicity and consistency	EFPIA suggests to replace 3rd bullet point by EFPIA proposal on sampoling strategies above. In addition, EFPIA suggests to add or refer to aligned narrative from end of chapter 4, line 326-334.
Alliance for Regenerative Medicine	812	814	Section 7.2.	"appropriate" is a subjective word.	A better phrase would be "risk- and science-based monitoring" or "performance-based monitoring" based on prior knowledge

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	820	820	7.2.	Major consensus topic: Continuous manufacturing This section states, that fluctuations in viral load during CM should be considered in the study design. This is not the case for conventional manufacturing (cf. section 6). We see no reason why this should be different for CM. Besides, determination of actual LRV using low virus concentration is not possible as virus concentration is reduced to below detection limit. The concept for virus clearance as described in Section 6 of this guideline (and elsewhere) relies on the assumption that virus clearance is independent on the concentration of virus. The only way to determine reduction factors is to spike with high concentration of virus and assessing the amount of virus before and after a processing step followed by calculating the Log Reduction Value (LRV). In conventional manufacturing, we trust the LRV to be valid irrespective concentration of virus.	viral load always reflects worst case independent of continuous mode of operation. Therefore, EFPIA suggests to delete "viral load".
EFPIA	822	822	7,2	Major consensus topic: Continuous manufacturing akwardly phrased.	EFPIA suggests to replace flow rate with residence time; make "temporal disturbance or pausing" a separate line
EFPIA	824	824	7,2	Major consensus topic: Continuous manufacturing Major: The industry already performs multi-column cycling and is not a new consideration point.	EFPIA suggests to replace "multi-column cycling" by "new loading strategies, e.g. multi-column cycling and serial loading".
Charles River Laboratories	826	830	7.2.1	Missing some comments/considerations for unpurified bulk harvest testing under CM conditions; see lines 330 - 334. It could be repeated in this chapter (7.2.1) or even expanded - e.g. by describing the meaning of a "sublot" in a CM mode	
EFPIA	827	827	7.2.1	Consider revising 'endogenous retrovirus' to 'endogenous virus' as CM risks do not just apply to retrovirus depending on the cell line in scope	Revise as "endogenous virus"

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	838	845	7.2.2	Major consensus topic: Continuous manufacturing The text indicates that simultaneous validation of connected unit operations can be performed only when all unit operations are to be validated. The requirements for validation of a single unit operation is not clear. What if two operations cannot be performed separately due to equipment but only one of them is known to contribute to viral clearance? This appears to be inconsistent with the text presented in Lines 805-807 accordingly as follows "The manufacturing process may be partially run in continuous or connected mode of operation and it is possible to use knowledge/experience of virus clearance study design based on batch processes for the evaluation of unit operation if suitable."	EFPIA suggests to delete subclause "..but only when…"
Charles River Laboratories	840	840	7.2.2		adding in the bracket another item: "different running conditions at the begin, the end, and in case auf pausing"
Charles River Laboratories	844	847	7.2.2	Not clear with respect to spiking procedure in case of connected validation. Is spiking of the start material of the first step required and clearance analyzed through both steps or is a seperated (additional) spiking requested for the second step?	
Alliance for Regenerative Medicine	846	847	7.2.2	What scenarios are being considered here?	
EFPIA	847	847	7.2.2	Major consensus topic: Continuous manufacturing Major: The phrase "conventional scale-down model" could be misinterpreted or become historically misunderstood	Replace : "conventional scale-down model" By: "conventional batch scale-down model"
Lonza	848	848	7.2.2	"Low pH/solvent detergent inactivation" to be replaced by "pH, Detergent and Chemical Inactivation" for the purpose of future proofing the document for other modalities and complex molecules.	Simply refer to pH and detergent inactivation or chemical inactivation of just 'inactivation technologies'
BioPhorum	848	848	7.2.2	"Low pH/solvent detergent inactivation" to be replaced by "Low pH/High pH/solvent detergent inactivation"	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	857	864	7	Major consensus topic: Continuous manufacturing Minor (clarification): "Process controls should be defined to allow for filter changes and post-use integrity testing while maintaining viral clearance capacity. This should include not interrupting the continuous process and allowing material diversion in the event of a filter failure". Can the document clarify further as to the intent of this statement for virus filtration? for example, does this mean the CM system should allow the diversion of potentially non-conforming material from the product steam in the event of a filter failure?	Align more closely with the intent per Line 815-817
Pall Life Sciences	858	860	7.2.2	"Validation as a batch process could be appropriate if settings of parameters which have impact on virus clearance do not vary beyond ranges tested in the virus clearance study (e.g., worst case setpoint)". In our experience and in discussions wioth regulatory agencies, this approach would not be permitted.	May be more appropriate to suggest that an end-user discusses their intentions to validate a virus fitration step in a continuous process with the regulatory agencies before performing the studies.
Asahi Kasei Bioprocess Europe S.A./N.V.	858	860	7.2.2	Feedstocks during virus filtration in continuous processes can include significant fluctuations of protein, salt, and buffer concentrations, and it has not be thoroughly investigated whether those fluctuations impact virus removal. It has not been demonstrated that it is sufficient to test virus removal using homogeneous feeds at the extremes of the fluctuations without undergoing the fluctuations at the magnitude and frequency observed in manufacturing.	We recommend to clarify that validation as a batch process should only be appropriate if the feedstock is homogeneous at the time it reaches the filter. Batch validation of a non-homogeneous feedstock should only be appropriate if all fluctuations in feedstock characteristics at the magnitude and frequency observed in manufacturing have been demonstrated to have no impact on viral clearance.
EFPIA	863	864	7.2.2	Major consensus topic: Continuous manufacturing Major: Prescribing normal and deviation operations may be premature and constraining.	Delete the last sentence line 862 to 864. At minimum ICH phrase should be a suggestion not prescription for CM process. We are able to pause as validated.
EFPIA	876	877	8	Misleading sentence because it currently implies that the design of the viral clearance studies defines the design of the production process. Furthermore, the development of a production process is usually aimed at ensuring an appropriate level of virus safety and not to achieve a maximum viral clearance. The more so since it cannot be defined what a "maximum viral clearance" is.	EFPIA suggests to revise as "appropriate virus clearance"
Albrecht Gröner	877	877	8	"… to achieve maximum viral clearance;" 'maximum' is not in line with (6) Evaluation and Characterisation of Viral Clearance Precedures, epecially line 426-427 as here it is stated "It is not necessary to evaluate or characterise every step of a manufacturing process if adequate clearance is demonstrated by the use of fewer steps."	Careful design of viral clearance studies using different methods of virus inactivation or removal in the same production process to achieve adequate viral clearance resulting in a appropriately high margin of virus safety.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Charles River Laboratories	879	879	9		Suggest to add further definitions for Parental cell bank // Pre-bank // LIVCA (when different than EOPC) // Non-endogenous virus (see table 1) // Purified bulk //in vivo // in vitro assay (as a general term) and differentiated from cell based unspecific screening assay versus cell based specific screening assay // molecular assays // independent study
EFPIA	879	879	9	Major: Add a definition for "Virus seed"	Suggested definition, taken from FDA Guidance on Cell Substrates (Feb 2010): Viral Seed: A live viral preparation of uniform composition (although not necessarily clonal) derived from a single culture process, aliquoted into appropriate storage containers, and stored under appropriate conditions.
EFPIA	879	879	9	Major Consensus Topic: Control Cells Testing Guidance Major: Add a definition for "Control Cells"	Suggested definition provided [modified from the text provided for virus vaccines within FDA Guidance on Cell Substrates (Feb 2010)]:Cells that are split off from the production culture and maintained in parallel under the same conditions and using the same reagents (e.g., culture medium) but without expression of the viral vector and/or addition of the helper virus, in order to perform tests on cells that have not been exposed to the viral vector or helper virus (which may interfere with some tests).
EFPIA	879	879	9	Major consensus topic: Document structure and consistency It will be helpful to add "sublot" in Glossary which was mentioned in section 4.	EFPIA consensus Jan 27, 2023: sublot mentioned in line 330-332 EFPIA believes it is desirable to align definition with ICHQ13
EFPIA	884	887	9	Major Consensus Theme: LIVCA and EoPC Terminology & Defintions Major: The definition states that age may be measured as either “Passage level” or as “Doubling level”. Passage level does not really make sense as an age measure as the passage level may be constant even if cells are actively growing. Also, passage does not really fit with the way cultivation is done i perfusion systems. Doubling level makes sense. But – traditionally cell age is measured in days, which will correlate with doubling level depending of cultivation conditions. I suggest the definition be rewritten “…. population doubling level or chronological time…” Ensure equivalent to LIVCA term to avoid confusion	Edit EOPC glossary definition to also include chronological age, per IVCA definition End of Production Cells (EOPC): Cells harvested (under conditions comparable to those used in production) from the MCB or WCB cultured to a passage level, population doubling time <<or elapsed chronological age>> comparable to or beyond the highest level reached in production. EOPC corresponds to cells at or beyond the limit of in vitro cell age.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	884	945	9	Major Consensus Theme: LIVCA and EoPC Terminology & Defintions It reads as if EOPC and UPB are the same matrix.	Make a better distinction between the two, if any
Alliance for Regenerative Medicine	884	884	9	What is the example? point is not clear	
EFPIA	890	894	9	Major Consensus Theme: LIVCA and EoPC Terminology & Defintions Minor: The term used in the guideline main text is LIVCA – the glossary should align to this terminology. The terminology "cells at the LIVCA" has been chosen. However, no definition for "LIVCA" is included in the glossary. There is a definition for "EOPC" even though this terminology is "not preferred".	Insert to Glossary: <<Limit of In Vitro Cell Age: Cells at or beyond the in vitro cell age.>> [broad EFPIA consensus to add suggested narrative]
PPTA	890	890	9	While "*in vitro cell age*" is explained, "limit of in vitro cell age" (LIVCA) is not explained although it is extensively discussed in Section 3	Please include "LIVCA" in the Glossary.
PPTA	890	895	9	Change the section order for "Inactivation" and put before of "In vitro Cell age"	
EFPIA	895	895	9	Major consensus topic: Document structure and consistency Suggest changing "inactivation" to "virus inactivation" to align with cirus removal definition.	Suggest changing "inactivation" to "virus inactivation" to align with cirus removal definition.
EFPIA	897	900	9	MCB is clearly defined as a "single pool of cells derived from selected cell clone". Although this document is for marketing is there any provision for the use of non-clonal cells (MasterWells) for early clinical phases? From a biosafety aspect, would they still be managed under the principles of the guidance?	
EFPIA	899	900	6	Major consensus topic: Prior knowledge/Protein-virus interactions Rational: virus-product interactions which negatively affect virus clearance are an exception to the rule based on current understanding. For example, inactivation and virus filtration conditions are chosen so that we operate on a plateau regarding virus clearance capacity, independent of virus-product interactions.	EFPIA Proposal: "If data for more than one product is available for the specific step, the effectiveness of virus reduction should be comparable in each case." (from EMA guideline on virus safety for IMPs, 398498)

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	901	903	9	Major: Regarding the definition of Master Virus Seed, suggest to complete the definition	Master Virus Seed (MVS): A master virus seed (stock, lot, or bank) is a preparation of a vaccine virus, helper virus, or viral vector from which all future production will be derived, either directly, or via Working Virus Seeds.
Charles River Laboratories	906	910	9	further clarification of "agnostic NGS", "broad NGS", and "targeted NGS". Targeted NGS can be further differentiated - targeted at the level of library preparation (amplicon, capture assay) or at the bioinformatics pipline	
EFPIA	933	937	9	Minor: Process robustness definition states that robustness may be one of two characteristics. Then -which one of the two is it?	EFPIA suggests to re-phrase line 934: "The term robustness is used to describe one or both of the two different characteristics: …"
EFPIA	940	941	9	Major Consensus Topic: Replication competent virus testing Major: Add a definition for Replication Competent Viruses (RCVs). Only mentionned in footnote g of table A-5.	Add in Glossary : Replication competent virus (RCV): Recombination of the viral vector with trans-complementing virus sequenses, leading to revert to parental or wild type phenotype.
EFPIA	943	945	9	Major Consensus Topic: Scope, Definition and Sample Matrices of UBH for Conti Manufacturing MAJOR: With the enlargement of scope of the guideline, the definition of the "Unprocessed bulk" should be enlarged as well to explain what an Unprocessed bulk should be for each of the products included in the scope of this guideline.	Replace the definition by: Unprocessed bulk when a recombinant protein is produced in an animal cell substrate (e.g. CHO): One or multiple pooled harvests of cells and culture media. When cells are not readily accessible, the unprocessed bulk would constitute fluid harvested from the fermenter. Unprocessed bulk when a recombinant protein is produced in a viral vector multiplied in a cell substrate (e.g. baculovirus/insect cells): One or multiple protein/antigen harvest prior to any purification step. Unprocessed bulk when a live viral vector is multiplied in a cell substrate (e.g. adenovirus, MVA vectors): One or multiple viral harvests prior to any purification step.
Alliance for Regenerative Medicine	944	945	9	Wording can be misunderstood	rephrase to "the unprocessed bulk would constitute fluid harvested from the fermenter"
EFPIA	955	956	9	The end of the sentence in line 956 seems to be missing: "In this guideline, intentionally introduced, non-integrated viruses such as EBV used to immortalise cell substrates or BPV" (end of sentence?)	"In this guideline, intentionally introduced, non-integrated viruses such as EBV used to immortalise cell substrates or BPV fit in this category"

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	955	956	9	Incomplete sentence?	suggest to complete sentence
ProPharma Group <Paul Joosten>	955	956	9	In this guideline, intentionally introduced, non-integrated viruses such as Epstein-Barr Virus used to immortalise cell substrates or Bovine Papilloma Virus.	Unclear sentence, why Bovine Papilloma Virus? Please consider rewording.
Albrecht Gröner	955	956	9	Verb missing in sentence "In this guideline, intentionally introduced, non-integrated viruses such as Epstein-Barr Virus used to immortalise cell substrates or Bovine Papilloma Virus."	
PPTA	956	956	9	Suggest to add "fit in this category"	"or Bovine Papilloma Virus fit in this category."
Pall Life Sciences	957	959	9	Helper viruses are usually defined as specifically enabling the production of another virus, e.g. AAV. This defnition is too broad.	Revise definition or change all text to have clarity over the definition of a baculovirus as a helper virus. Currently defined as specifically not one in Annex 7.
EFPIA	957	959	9	Major Consensus Topic: Helper Virus Description/Defintion Major: Inconsistent nomenclature helper virus vs protein expression vector: Throughout most of the guideline, helper virus is used to refer to both 'actual' helper virus (i.e. adeno or herpes-simplex used to produce AAV) but also to 'protein expression vectors' (i.e. baculovirus). In two instances however (1338 and 1421), a more correct nomenclature is used, where the baculoviruses are referred to as protein expression vectors and NOT as helper virus . This is better, but the rest of the guideline is now inconsistent, and this new term is also not included in the glossary. Note: follow up discussion aims to address the consistency through the glossary definition for helper virus [Furthermore, EFPIA recognise that clear guidance is provided on adventitious and helper viruses. Expectations for a third category, protein expression viruses (i.e. baculovirus, as defined in lines 1338), is unclear. We assume that baculoviruses are in many cases intended to be covered by the term helper virus, this is however not clear in addition to being scientifically incorrect. It is also not clear if 'helper virus' is intended to cover only baculoviruses used to produce viral vectors, or also baculoviruses used to produce recombinant proteins (which may have different requirements in certain regards). This choice of nomenclature is very confusing and leaves a lot of unclarity regarding the requirements for protein expression vectors.]	Proposal to consistently use protein expression vector for baculoviruses: •Add protein expression vector to the glossary •Align wording in 16 - 22 with correct wording in 1339-1341 •Update from 'helper virus' to 'helper virus or protein expression virus' in several instances where both are meant. EFPIA Proposal is to revise the text throughout the guideline to consistently use the nomenclature 'protein expression vector' for referring to baculovirus: • Line 20 – 22: Furthermore, the scope includes Adeno-Associated Virus (AAV) gene therapy vectors that depend on helper viruses such as baculovirus, herpes simplex virus or adenovirus for their production, or use a protein expression vector such as baculovirus in the production. • Update from 'helper virus' to 'helper virus and/or protein expression virus' in several instances where both are presumably meant: line 69, 85, 409, 410, Table 4 (including footnote 9), table A-5, 1348, 1352, 1370, Table A-5 (including footnote f), 1427, 1440-1442 • Line 1339-1341: Helper-virus dependent products require a helper virus to enable expression of the viral vector (e.g., adeno-associated virus that are expressed using a helper virus such as herpes simplex virus or

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	957	959	9	Major Consensus Topic: Helper Virus Description/Defintion Major: Inconsistent nomenclature helper virus vs protein expression vector: Throughout most of the guideline, helper virus is used to refer to both 'actual' helper virus (i.e. adeno or herpes-simplex used to produce AAV) but also to 'protein expression vectors' (i.e. baculovirus). In two instances however (1338 and 1421), a more correct nomenclature is used, where the baculoviruses are referred to as protein expression vectors and NOT as helper virus . This is better, but the rest of the guideline is now inconsistent, and this new term is also not included in the glossary. Note: follow up discussion aims to address the consistency through the glossary definition for helper virus [Furthermore, EFPIA recognise that clear guidance is provided on adventitious and helper viruses. Expectations for a third category, protein expression viruses (i.e. baculovirus, as defined in lines 1338), is unclear. We assume that baculoviruses are in many cases intended to be covered by the term helper virus, this is however not clear in addition to being scientifically incorrect. It is also not clear if 'helper virus' is intended to cover only baculoviruses used to produce viral vectors, or also baculoviruses used to produce recombinant proteins (which may have different requirements in certain regards). This choice of nomenclature is very confusing and leaves a lot of unclarity regarding the requirements for protein expression vectors.]	The following updates are also proposed to the Glossary: •Helper virus: A virus that provides helper functions allowing an otherwise replication-deficient coinfecting virus to replicate. In the context of this guidance, this generally refers to an adenovirus or herpes simplex virus that is used in the production process of an AAV product. •Protein-expression vector: A recombinant virus, such as a baculovirus, that is used to express a recombinant protein or a viral vector such as AAV. •Viral vector-derived product: A product encoded and expressed by a recombinant virus, where the recombinant virus is referred to as a protein expression vector, such as a baculovirus. •Viral vector: A recombinant virus that may be applied in vivo as a medicinal product or applied ex vivo for other advanced therapeutic applications. The genetically engineered viral vector may require a helper virus for production, or may use a protein expression vector such as baculovirus for production.
PPTA	959	959	9	Suggest to revise as reported in column G	"…or replication of the product viral vector."
Asahi Kasei Bioprocess Europe S.A./N.V.	973	975	9	The definition provided for "Virus-like Particles" provides only an empirical description of the particles rather than a technical definition of the structure of the particles.	We recommend providing a technical definition of "Virus-like Particles", such as "Particles having virus-like structures that self-assemble as a result of the expression of proteins encoding capsids, cores or envelops of viruses"
Asahi Kasei Bioprocess Europe S.A./N.V.	978	981	9	The definition of "viral vector" is not accurate. Since the definition of "Virus" is an "Intracellularly replicating infectious agent" (line 947), and since many viral vectors are not replicating, it is not appropriate to define all viral vectors as "recombinant virus".	We recommend to use another term such as "recombinant virus-like particles" rather than "recombinant virus".
ProPharma Group <Paul Joosten>	986	987	9	The WCB is prepared from aliquots of a homogeneous suspension of cells obtained from culturing the MCB under defined culture conditions.	Please consider if a WCB can also be derived from another WCB
ProPharma Group <Paul Joosten>	988	989	9	Working Virus Seed (WVS) A working virus seed (stock, lot, or bank) is produced from the MVS.	Please consider if a WVS can also be derived from another WVS.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	990	990	Table 1	Minor Shared Theme: Retrovirus testing for cell lines at Table 1 Major: Tests for Retroviruses at MCB + LIVCA for cell lines not known to produce retroviral particles. Table 1: Infectivity test is listed as + for MCB and LIVCA. Concern: Infectivity should not be required for cell lines not known to produce retroviral particles (like HEK293), unless retrovirus is detected by electron microscopy or PERT. This is now described accurately in Section 3.2.1, but in this table it looks like it is always required for all cell lines	Insert clarification footnote for infectivity test at Table 1 : <<For cell lines not known to produce retroviral particles, infectivity is normally only required in case of a positive TEM or RT result">>
EFPIA	990		Table 1	Major Consensus Topic:Further advocacy to limit application of in vivo testing Major Need to perform In vivo not aligned with Section 3.3.2 or with the footnote	For MCB/LIVCA, propose to change "+" to "(+)"
EFPIA	990	1013	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 MAJOR: Another table is proposed (see attached) - Test for retroviruses and other endogeneous virus is updated to consider the two cases described in section 3.2.1: if the cell line is not known to produce retroviral particles, or if the cell line is known to produce retroviral particles. - Better highlight the use of broad molecular method (NGS), these methods should be proposed at the same level than the other methods, and not only as a replacement of other methods. - Footnotes simplified and replaced by cross-references to the specific sections where the methods' specifities are already described to avoid duplication and sometime misalignement between the footnote and the section. See specific comments on the footnotes below.	See the file of proposed Table 1 [to help address the repeat queries for more clarity in table 1, such as the meaning of "+", (+) and to help consolidate the increasing # footnotes, EFPIA propose an suggested alternative table 1 layout in a separate attached file (word doc). Within the table, the testing requirements are provided alongside where risk based cosiderations apply, and the assoiciated footnote retained, but as a cross reference to the relevant narrative sections associated with the test].
EFPIA	990	990	Table 1	Minor Shared Theme: Retrovirus testing for cell lines at Table 1 Table 1: Infectivity test is listed as + for MCB and LIVCA. Text appears to apply to all cell lines but does not consider cases where either cell lines do not produce RVLP or when there is a negative TEM or RT result.	Recommend to modify to clearly articulate the cases where Infectivity assay is required based on risk assessment or other supporting data (ex. positive TEM or RT result) to be consistent with the recommendation in Section 3.2.1, lines 170-186.
EFPIA	990	990	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Table 1: In vivo assays or NGS, footnote g. Recommend to simplify the decision process for when in vivo tests as the footnote is quite extensive and complicated.	Consider incorporation of text from 3.2.1. footnote g in the main body of the document and declare only the limited cases where in vivo testing would be required (ex. novel cell substrates).

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	990	990	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 We recommend clarity and alignment between footnotes “g” and “f” and the content in the text.	
EFPIA	990		Table 1	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies Testing of LIVCA cells is dispensable for well-characterized cells since it is unlikely that these cell banks harbor any virus that may remain undetected, particularly if broad virus detection methods, such as NGS, are used for cell bank testing. Testing of each unprocessed bulk is considered adequate to ensure virus safety.	
EFPIA	990	990	Table 1	Minor: footnote B is not fully in line with earlier definitions "cells at the limit of in vitro cell age used for production" implies at harvest and earlier definitions include beyond	Suggest truncating and just cross ref to section 3.1.3
EFPIA	990		Table 1	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies Delete footnote f for in vitro assays for MCB?	
EFPIA	990		Table 1	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies See comments for line 121 – 124, section 3.1.2. Discrimination between test requirements for initial and subsequent WCB or, alternatively, between WCBs used for LIVCA and WCB not used for LIVCA should be indicated. If necessary, footnote f should be adapted accordingly.	
EFPIA	990		Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 In alignment with WCB and LIVCA, why no footnote with reference to section 3.1.1.?	
EFPIA	990		Table 1	Major Consensus Topic: LIVCA and EoPC Terminology & Defintions Please add a definition of the various terms (LIVCA, EOPC, ECB) in the Appendix. Clarification is requested accordingly since EOPC PDL could differ depending on passaging scheme and test results ultimately used to establish a LIVCA	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
SGS Vitrology Ltd	990	990	Table 1	To align with rationale that testing for retrovirus is determined on the cell line and if it is know to produce retroviral particles or not. Line 170-178: Cell lines not known to produce RVPs require TEM and PCR-based RT; with infectivity required on the event of confirmation of positive RT or positive TEM	Should "Infectivity" testing at MCB and LIVCA stage include a footnote on requirement as appropriate?
ProPharma Group <Paul Joosten>	990		9	Table 1. Virus Tests Recommended to Be Performed Once at Various Cell Levels	Please consider Various Cell Passage Levels.
PPTA	990	1048	Tables 1 to 4	Tables 1 to 4 are part of the "Glossary" (as in Q5A(R1)) –which is not comprehensible when considering the actual meaning of the term "Glossary"	Proposal to shift Tables 1 to 4 into a separate Annex.
Alliance for Regenerative Medicine	990	991	Table 1		Suggest to list the virus types irrespective of origin e.g retroviruses, other viruses
Alliance for Regenerative Medicine	990	991	Table 1	Suggest to list the virus types irrespective of origin e.g since retroviruses might be adventitious viruses. Also states: "f. The in vitro virus test is performed directly on the WCB or on LIVCA cells directly derived from this WCB. Tests for viruses using broad molecular methods (NGS) can be used as supplementary or replacement assays for the in vitro tests (cell culture and PCR) based on the risk assessment." Its unclear what the expectation for in vitro testing is when there is no WCB i.e. only an MCB. Suggest to revise for clarity.	To avoid confusion, suggest to use a third indication for testing where there are options e.g. (+). So in vitro or NGS, vivo assays or NGS and antibody production tests or specific molecular assay would state (+) with footnotes f), g) and h) respectively to clarify this. And revise footnote to e.g. "f. The in vitro virus test is performed directly on the WCB or on LIVCA cells directly derived from the MCB or WCB. Tests for viruses using broad molecular methods (NGS) can be used as supplementary or replacement assays for the in vitro tests (cell culture and PCR) based on the risk assessment."
PTC Therapeutics	990	1015	9	Table 1 – infectivity: can be clarified by adding a footnote that for cell lines not known to produce retroviruses, this test is only needed if RT assay comes positive, else this is not required. This part has been clarified now in section 3.2.1 – Test for retroviruses	Addition of footnote "for cell lines not known to produce retroviruses, this test is only needed if RT assay comes back positive, otherwise, this test is not required."
PTC Therapeutics	990	1015	9	Table 1 – re: in vivo and in vitro virus tests. The guidance says nucleic acids tests can be used instead of in vitro and in vivo tests based on risk assessment. An idea of minimum panel of viruses that needs to be looked at would be helpful.	
EFPIA	992	992	Table 1	Omit "b. Cells at the limit: "	See column F

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	992	992	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Minor comment: consider aligning the format of Table 1 and Table A.5	See column F
EFPIA	992	992	Table 1	Delete repeated "Cells at the limit:".	Delete repeated "Cells at the limit:".
EFPIA	993	993	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Content of footnotes c should be added to section 3.2.1 and the footnotes remove.	Remove footnote c. See the proposal in the attached file.
EFPIA	994	995	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Content of footnotes d and e is already capture in Section 3.2.1, suggested to cross-reference to the section and remove the footnotes.	Remove content of footnote h and replace it with a cross-reference to Section 3.2.1. See the proposal in the attached file.
EFPIA	994	994	Table 1	Minor Shared Theme: Retrovirus testing for cell lines at Table 1 See comment for lines 179 – 181, section 3.2.1 and based on outcome adjust footnote accordingly.	
Charles River Laboratories	996	998	Table 1	Footnote f: the wording and relevance is unclear. One could think in vitro testing on MCB is not required but only on WCB or cells at LIVCA (seems the second sentence has relevance for MCB)	Suggestion (first sentence): " The in vitro virus test is performed directly on the MCB and on the WCB or on LIVCA cells directly derived from this WCB"
EFPIA	996	998	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Footnote f is not aligned with what is proposed in Table 1. On the table, the in vitro assay is proposed on the MCB, WCB, and LIVCA. In the footnote, the in vitro assay is proposed on the WCB or the LIVCA.	To be clarified. See the proposal in the attached file.
EFPIA	996	998	Table 1	Let the door open to other broad molecular methods We would like to propose the term "broad molecular methods" instead of "NGS" as alternative (in attached Table 1). And mentioned NGS as an example in brackets. EWG considered NGS as the only example broad detection method currently. This is also the way how NGS is presented in paragraph "3.2.3 In Vivo Assays." We find this proposal to be limited to the current view of technology and too restrictive.	Table 1: Add: ""The in vitro virus test is performed directly on the WCB or on LIVCA cells directly derived from this WCB. 996 Tests for viruses using broad molecular methods (e.g., NGS) can be used as supplementary or replacement assays 997 for the in vitro tests (cell culture and PCR) based on the risk assessment"

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
PPTA	996	996	Table 1	"*The in vitro virus test is performed directly on the WCB or on LIVCA cells directly derived from this WCB* ." Table 1, footnote 'f' is also applicable to MCB yet 'MCB' not listed in footnote line 996	Include also MCB into Footnote "f": "*…directly on the MCB, WCB or on LIVCA cells…* "
EFPIA	997	998	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Could be deleted because content already covered by footnote j in lines 1014 – 1015.	
Lonza	999	1000	Table 1	The statement 'However, in vivo testing is not necessary for well-characterised cell lines such as CHO, NS0 and SP2/0, based on cell line history' is contradictory to the FDA guideline for industry, *Characterisation and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications* and the WHO guideline *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks, Annex 3, TRS No 978. Or the China regulation applied prior to ICH sign up.* How will global acceptance across the ICH countries will be managed. Is there a work stream looking at the overall impact on other regulatory documents (as per the comment above on EMA 398498).	Reference to a company's risk based approach should be described here in light of potential moves toward less in vivo testing which is the general trend based upon the availability of other newer technologies in the field of biosafety testing. Acknowledging this movement or trend here might go some way to addressing any contradiction in other currently established guidance or references.
Charles River Laboratories	999	1009	Table 1	Footnote g: This is a bit unclear. Does it allow omission of in vivo testing for CHO, NSO and SP2/0 cell lines without risk assessment?	If this is the understanding I suggest to add a specific chapter under chapter 3 to outline application of "Prior Knowledge" in testing strategies similar to chapter 6.6 outlining prior knowledge application for viral clearance
EFPIA	999	1009	Table 1	Major Consensus Topic:Further advocacy to limit application of in vivo testing Footnote g. First, can one say that a cell line is well-characterized, particularly when dealing with adventitious agents? The terminology "well-characterized" is applicable to the DS or DP stage, but not on a cell line. Second, the viral safety risk is linked, of course, on the control of the cell line at previous stages, but also at the microbial quality of the raw material used, and of the environement. Therefore, in all cases (CHO, NS0, and SP2/0 or other cell lines), the selection of tests to be performed should be based on a risk assessment. The risk assessment should be revised in case of change of manufacturing process, and the testing profile updated if needed. This footnote is not aligned with section 3.2.3 In vivo assay.	Remove content of footnote g and replace it with a cross-reference to Section 3.2.3. See the proposal in the attached file.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
BioPhorum	999	1000	Table 1	The statement 'However, in vivo testing is not necessary for well-characterised cell lines such as CHO, NS0 and SP2/0, based on cell line history' is contradictory to the FDA guideline for industry, Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications and the WHO guideline Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks, Annex 3, TRS No 978. This should therefore be addressed not as a footnote to a table but as a paragraph and should clarify why the new recommendation superseeds key documents commonly used by industry and how global acceptance accross the ICH countries will be managed	
EFPIA	1001	1002	Table 1	Major Consensus Topic:Further advocacy to limit application of in vivo testing Clarification on the risk based approach to testing is requested (ex. if Parental Cell Line is tested does this remove the testing requirement for MCB or is this an additional testing requirement)? Major Consensus Topic: LIVCA and EoPC Terminology & Defintions	
Charles River Laboratories	1010	1012	Table 1	Footnote h: The MAP/HAP/RAP assay is a specicies specific assay (for rodent derived material) and can be regarded a virus specific test and could be coverd in the table under the row "other virus specific tests". (see also to Annex 7, table A-5, footnote d; line 1384-1385)	the related row in table 1 could be removed and the MAP/HAP/RAP assay indicated in footnote i related to "other virus specific tests"
EFPIA	1010	1012	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 The content of footnote h. is more or less a duplication of section 3.2.4.	Remove content of footnote h and replace it with a cross-reference to Section 3.2.4. See the proposal in the attached file.
EFPIA	1010	1012	Table 1	Major Consensus Topic: Potential WCB and LIVCA Test Point Redundancies Footnote h: Why are antibody production tests still required for well characterized cell lines such as CHO? Requirement for antibody production tests should be analogous to in vivo testing (footnote g) based on cell line history, prior knowledge (testing of parental cell bank) and other risk-based considerations.	
EFPIA	1010	1012	Table 1	Major Consensus Topic:Further advocacy to limit application of in vivo testing The principles of substitution of in vivo testing and a risk based approach for the testing program should be consistently applied in the revised guideline. This applies to the following topics: With regard to master cell bank and virus seed characterization, testing for antibody production should be abandoned and deleted from Tables 1 and A-5. Relevant virus contaminants should be covered by "other virus-specific tests" focusing on complementary methods, such as NATs or NGS.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1010	1012	Table 1	Minor and Shared Comment: Genericise the NAT methods applicable to replace Ab Production Tests Why differentiation between virus-specific PC or targeted molecular methods? The former falls also under targeted molecular methods.	
EFPIA	1013	1015	Table 1	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Remove the content of the footnotes and cross-reference to the specific sections.	See the proposal in the attached file.
Charles River Laboratories	1014	1015	Table 1	Footnote j: footnote "j" should be added to the table under *Other virus specific tests* as the footnote refers to these cells too	
EFPIA	1014	1015	Table 1	Minor: Footnote j indicates: "When applicable, NGS (...) may be used to replace (...) other virus specific tests based on assay suitability and risk assessment". However, the "j" is not mentioned next to "other virus specific tests" in the table 1 above.	Last line of Table 1: "other virus specific tests (i) (j)".
EFPIA	1014	1015	Table 1	Major Consensus Topic: More clarity on options to substitute IVV with targeted NAT Major NGS only indicated as alternative to In Vitro virus assay.	In Section 3.3.2, other NATs are also mentionned as alternative. Propose to write In vitro or NGS/NAT in Table 1
PPTA	1014	1015	Table 1	"When applicable, NGS should be considered to replace the in vivo test and may be used to supplement or replace the in vitro and other virus specific tests based on assay suitability and risk assessment." In Table 1, Footnote "j" is also indicated to be applicable to the "Antibody production tests or specific molecular assay", however this test is not mentioned in Footnote "j"	Please add further clarification whether footnote 'j' is intentional for 'Antibody production tests or specific molecular assay'.
ViruSure GmbH- Andy Bailey	1016	1016	Table 2	The study of Gombold et. al. (2014) confirmed a limited ability of the in vivo system to detect a range of potential virus contaminants.	For "in vivo virus screen" change the "Detection Capability" to "Limited rand of viruses"
Charles River Laboratories	1016	1018	Table 2	Antibody Production:	Suggestion for replacement: Detection Limit: Viruses failing to replicate and/or produce anibodies in the applied animals under protocol conditions
Charles River Laboratories	1016	1018	Table 2	TEM on:	Suggestion: detection capability: viruses and virus like particles with assessment of identity // Detection limit: Qualitative or quantitative assay with low sensitivity

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1016		Table 2	Minor Shared Comment: UBH sample matrix types for testing at Table 2 Major Guidance on sample preparation for virus testing is missing: e.g. how many freeze/thaw cycles (WHO TRS 978 Annex 3 recommends 3 F/T cycles, with separate storage of primary supernatant)	
EFPIA	1016		Table 2	Minor Shared Comment: UBH sample matrix types for testing at Table 2 Guidance needed for unpurified BH when lysate/supernatant is required or simple unpurified harvest without enhancing virus detection by release of intracellular virus	Please clarify, if and when unpurified BH could be used
EFPIA	1016	1016	Table 2	Major Consensus Topic:Further advocacy to limit application of in vivo testing Major: In vivo virus screen In the detection limitation column, the reference to Gombold et al should be added to show the limited value of the tests in animals	Reference to the scientific article below should be added: Systematic evaluation of in vitro and in vivo adventitious virus assays for the detection of viral contamination of cell banks and biological products. James Gombold, Stephen Karakasidis, Paula Niksa, John Podczasy, Kitti Neumann, James Richardson, Nandini Sane, Renita Johnson-Leva, Valerie Randolph, Jerald Sadoff, Phillip Minor, Alexander Schmidt, Paul Duncan, Rebecca L. Sheets. Vaccines 32 (2014) 2916-2926. https://doi.org/10.1016/j.vaccine.2014.02.021
EFPIA	1016	1017	Table 2	Minor Shared Comment: UBH sample matrix types for testing at Table 2 Major: In vitro virus screen for: "2. production screen" can be done on "Unprocessed bulk harvest or lysate of cells and their cell culture medium from the production reactor". Those two test article are the same thing.	Proposal to replace by: "Unprocessed bulk harvest (see section 4)" or lysate of cells and their cell culture medium from the production reactor"
EFPIA	1016	1017	Table 2	Major: In vitro testing for bank characterization and production screen can be done on lysates of cells and their culture supernatant. If "UPB" and " lysate of cells and their cell culture medium" are considered the same, can in vitro testing results of the UPB be used for LIVCA determination?	In cases where the UPB and EOPC are the same matrix, specify that testing results of the UPB can be used for LIVCA determination in order to avoid unnecessary testing redundancy
EFPIA	1016	1016	Table 2	Minor: For in vitro virus screen: Viruses failing to replicate or produce diseases in the test system	Proposal to remove "or produce diseases" for in vitro test And replace by 'observable effects'

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
ProPharma Group <Paul Joosten>	1016		9	Table 2. Antibody Production Lysate of cells and their culture medium Specific viral antigens Antigens not infectious for animal test system	Please consider adding that not all virusinfections result in a measurable antibody response.
PPTA	1016	1016	Table 2	Column: "DETECTION LIMITATION", Line "*in vitro* virus screening": "*Viruses failing to replicate or produce diseases in the test system*"	Proposal to change to "*...produce signs of infection in the test system*"
Alliance for Regenerative Medicine	1016	1016	Table 2	Broad Screen Molecular methods: Positive result does not indicate whether virus is infectious and may require confirmatory testing Not sure this is accurate statement, depends on the type of testing done by NGS. If mRNA is used this is a very strong indication that the virus has infected those cells and is being produced?	Suggest to provide clarification regarding consideration of the capability of the analytical procedure to detect and/or quantify an infectious virus(es).
BioPhorum	1019	1020	Table 3	Thymic virus. There are discrepancies in literature over the sequence for thymic virus which could benefit from clarification when using a PCR based method. Please add a clear definition.	
PPTA	1019	1019	Table 3	Column: "HAP": footnote(s) for SV5 are missing	Add footnotes 1-3, as appropriate
Charles River Laboratories	1024	1025	Table 3	see comment to line 228-231	allowing replacement of the antibody production assay by both targeted or non targeted/agnostic molecular methods
Charles River Laboratories	1027	1028	Table 4	"Virus like particles": Case C and D do not exclude the presence of virus particles	should be "+"
EFPIA	1027	1028	Table 4	What is the difference in meaning between +-sign and +-sign in brackets?	
EFPIA	1027	1028	Table 4	Revise title of table to create clarity and link to page 16 where it is referenced.	Proposal: "Recommended Action Plan in Response to the results of virus tests on cells or unprocessed bulk."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1027	1027	Table 4	Major consensus topic: Document structure & consistency Lines 611-614 of the track changes version or lines 477-478 of the clean version describe the use of Retroviral-Like Particles for these studies. Since this is described in the text, it should also be in Table 4 for additional clarity.	Amgen recommends adding the text from lines 611-614 to the table here under "ACTION" as a method of assessment: "For CHO cell-derived products, CHO-derived endogenous virus particles can also be used for viral clearance experiments. There is no infectivity assay for these particles, and the detection assay (e.g., molecular or biochemical) should be qualified for its use."
EFPIA	1027	1048	Table 4	For Case B, Test for virus in purified bulk, it states "no" in this table, however, it is inconsistent with section 5 Case B where "no" only applies for cell lines such as CHO, C127, BHK and murine hybridoma cell lines which were mentioned from line 375 to 380. For other rodent cell lines, purified bulk should be tested.	Suggest changing "No" in Table 4 to "Yes/No" and add a footnote referring to section 5 Case B for specific requirment for different cell lines.
EFPIA	1028	1028	Table	Table 4. Not clear what the ( ) mean in this table, where there is (+) vs +	Add a footnote to explain what ( ) means
SGS Vitrology Ltd	1028	1028	Table 4	To align with rationale that testing may/may not be required based on the cell line. Lines 372-374: Purified bulk should be tested using suitable method…..Lines 375-380: Cell lines such as CHO, C127, BHK…..it usually is not recommended to test….in the purified bulk or drug substance	Should "Test for virus in purified bulk" under Case B include a footnote on requirement as appropriate?
ViruSure GmbH- Andy Bailey	1033	1034	Table 4	"where" would fit better with the context of the sentence	Possible type: "whether" or "where"
PPTA	1044	1044		…"*If this is not possible, then a specific model virus should be used.). When...* "	Change to "*If this is not possible, then a specific model virus should be used.). When…* "
Albrecht Gröner	1047	1048	Table 4	Sensitivity of assay to detect viruses (here helper viruses) in purified bulk is not high enough to document sufficient safety margin (compare line 25 of this Comment file and embedded Excel file "Virus Safety of Purified Bulk")	delete sentence and refer to an appropriate margin of virus safety
EFPIA	1049	1073	Annex 1	We should discuss (again) whether we would suggest to delete this section. I know of nobody producing medicines using ascites technology. It is an outdated technology. In line with the general transition into animal-free production systems, the use of animals for ascites production should be a ting of the past. It is a dinosaur.	Delete annex 1
Charles River Laboratories	1070	1073	Annex 1	this is not consistent with other chapters. E.g. why are "cell based unspecific screening assay" `(in vitro/3.2.2) or embryonated eggs excluded, and why isn't replacement or supplement by molecular methods like NGS or other advanced assays allowed?	Suggest to reference chapter 3.2 as applicable

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1072	1073	anexe 1	Major: the testing to be performed should be based on risk assessement and using most apropriate methods. In vivo assays have been demonstrated to be restricted in range and low in detection limits (Gombold et al 2014). Most appropriate in vitro assays exists including cell based and molecular biology based assays	replace "and perform species-specific assay(s) as well as in vivo testing in adult and suckling mice". By "and perform species-specific assay(s) as per risk assessement using the most appropriate in vitro assays or in vivo assay if properly justified
ViruSure GmbH- Andy Bailey	1075	1082	Annex 2:A	Reovirus has become a widely used model virus for the validation of CHO derived therapeutics, and along with MuLV, MMV and PRV, this panel of 4 viruses is often selected. This panel though would not fit with the criteria listed in this section, and althout the statement is included that these are only examples, to avoid confusion it would be recommended to include an option with Reovirus.	Include a scenario with Reovirus as one of the model viruses
Charles River Laboratories	1077	1078	Annex 2		suggest to indicate parvoviruses specifically and removing SV40. Parvoviruses are recognized generally as most challenging viruses; SV40 is specific to some level and "resistance" can be different between different strains or when cultured differently (in house data, not published) - not an ideal model why it shouldn't explicitely be mentioned here anymore
PPTA	1077	1077	Annex 2	SV40 is quite a large virus to be using as a non-enveloped virus for e.g. filtration, recommending parvoviruses for filtration would be better	
BioPhorum	1081	1081	Annex 2	Could add more examples and replace with (e.g., HSV-1, SuHV-1, or a pseudorabies virus). Current description does not reflect that parvovirus is the virus of choice for industry	
Pall Life Sciences	1091	1092	Annex 2	"Generally, the process should be assessed for its ability to clear at least three different viruses with differing characteristics." Is this from two independent studies? From one lot of product?	Define further

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Regeneron Pharmaceuticals, Inc.	1091	1092	Annex 2.B	"Generally, the process should be assessed for its ability to clear at least three different viruses with differing characteristics." This sentence indicates a minimum of three different model viruses should be used to evaluate product specific viral clearance for a process. A minimum three viruses may not be necessary when relevant prior knowledge with diverse viruses exists for robust steps (e.g., low pH viral inactivation, virus-retentive filtration). As suggested in Annex 6: Examples of Prior Knowledge Including In-House Experience to Reduce Product Specific Validation Effort (L1200 - 1324), the prior knowledge could allow fewer than three viruses to be evaluated. Evaluating more model viruses than what is necessary is burdensome with minimal benefit because it requires additional time and resources to complete and potentially delays the time for development of novel medicines to treat unmet medical needs. Depending on the scope of the validation effort, each additional model virus introduces new challenges, including the potential to double the time for assay development and validation, and personnel needed or allocated to perform the study. Moreover, the information gained by this requirement would be of limited value if prior knowledge can be applied. Furthermore, this above referenced sentence conflicts with a sentence in the section 6.1.1 (L491 - 493): "The choice and number of viruses used should be influenced by the quality and characterisation of the cell lines and the production process." The sentence in L1091 – 1092 (referenced above) indicates that at least three different model viruses should be used during viral clearance evaluation of a process. However, the sentence in L491 – 493 suggests that the number of model viruses is flexible, and that it should depend on knowledge of the cell line and production process. The inclusion of these two conflicting sentences in separate areas of this guideline may lead to divergent interpretations regarding the acceptable minimum number of model viruses.	Regeneron requests that this sentence in L1091 - 1092 explicitly align with Annex 6: Examples of Prior Knowledge Including In-House Experience to Reduce Product Specific Validation Effort (L1200 - 1324) of this same guideline. We propose the following revision to L1091 - 1092 to enhance flexibility: "The choice and number of viruses used for evaluation of the process should be influenced by relevant prior knowledge. Generally, the process should be assessed for its ability to clear at least three different viruses with differing characteristics. Application of prior knowledge should inform the number of viruses assessed by product specific studies." This alignment would reduce divergent interpretations of guideline on the acceptable minimum number of model viruses and provide flexibility based on sound quality risk management.
Octapharma Biopharmaceuticals GmbH	1091	1092	Annex 2 B	Important information should be incorporated in main text: 3 viruses to be tested at least.	
Alliance for Regenerative Medicine	1091	1092	Annex 2	Generally, the process should be assessed for its ability to clear at least three different viruses with differing characteristics.	This type of information would be useful in the main text (Section 5 or 6).
Albrecht Gröner	1092	1092	Annex 2	for clarification	Generally, the process should be assessed for its ability to clear at least three different viruses, including retroviruses / retrovirus-like particles, with differing characteristics.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
ViruSure GmbH- Andy Bailey	1093	1093	Table A-1	Formatting	Formatting suggestion: References to the footnote in the table could be superscripted as in the other tables to ease finding the points use in the table
EFPIA	1093	1093	ANNEX 2	Add a column with the pI of the different model viruses	EFPIA suggests to add a column with the pI of the different model viruses
SGS Vitrology Ltd	1093	1093	Table A-1	Typographical error	Virus name update - autographa californica multiple nucleopolyhedrovirus
SGS Vitrology Ltd	1093	1093	Table A-1	Typographical error	Should Vesivirus 2711 be Vesivirus 2117?
ProPharma Group <Erik Schagen & Kristiena Abbink>	1093	1101	Annex 2	Table A-1. References to footnotes are indicated as regular characters.	Suggest to adjust the references to superscript for clarity.
EFPIA	1129	1129	Annex 3	Capitalize "S"	See column F
Octapharma Biopharmaceuticals GmbH	1129	1129	Annex 3	Typo S and s in text and formula	
Albrecht Gröner	1156	1157	Annex 4	Compare Line 17 of this Comment file	… of the virus load in the pre-processed material and the virus load in the post-processed material ….
Albrecht Gröner	1168	1168	Annex 4	Compare Line 17 of this Comment file	…. after the process step.
EFPIA	1182	1182	Annex 5	Major consensus topic: Document structure & consistency Consistency & scientific accuracy. Does not matter where testing is done as long as testing is done before the claimed purification process. Replace "cell culture harvest" by virus which may be entering the purification process as described in line 431	Proposal: "Measured or estimated concentration of virus which may be entering the purification process"
PPTA	1190	1190	Annex 5	Remove "l"	<106 particles/dose l
Octapharma Biopharmaceuticals GmbH	1190	1190	Annex 5	Typo: dosel	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Pall Life Sciences	1197	1199	Annex 5	"In the Case B scenario for Chinese Hamster Ovary (CHO) cells, a safety margin of <10-4 particles/dose is considered acceptable for Retroviral-Like Particles (RVLPs) for recombinant proteins if in vitro testing fails to identify the presence of infectious retroviruses". Please include rationale for the safety margin of <10-4 particles/dose	
Lonza	1197	1199	Annex 5	Replace CHO cells with recombinant proteins expressed from mammalian cells. This future proofs the doc and is less prescriptive.	
Lonza	1197	1199	Annex 5	The safety margin of less than 1 x 10 E4 should be explained so that there is a clear understanding of what this is measuring, what equivalence is to demonstrated through this value as applied to other modalities & non mAb processes. It needs to be put in the overall context of demonstrating no / reduced risk of harm to the patient. Closed processing for example is a mitigation for not being able to demonstrate viral clearance.	A number for the traditionally performed dose risk calculation is now specified as a guide but no similar such number is provided for modalities outside traditional biologics from CHO. Is a number required and if not possible what is the equivalent approach for other modalities. For example mitigation by additional testing or closed processing.
Charles River Laboratories	1197	1199	Annex 5	A: maybe the symbol <10-4 should be replaced by ≤ 10-4 B: The final portion of the sentence ("…..if in vitro testing fails to identify the presence of infectious retroviruses") is a bit difficult to understand The "in vitro" testing is probaly the retrovirus infectivity assay performed on the MCB/LIVCA. Maybe that should be clearly said (in case I understand this corrcetly)	Suggestion: "…..…….if retrovirus infectivity testing fails to identify the presence of infectious retroviruses in MCB and LIVCA"
BioPhorum	1197	1199	Annex 5	4-log safety factor acceptable, could be made more visible - will be benefitial to be in the main guideline	
BioPhorum	1197	1199	Annex 5	Replace CHO cells with recombinant proteins expressed from mammalian cells to widen the examples for which the approach is deemed acceptable.	
BioPhorum	1197	1199	Annex 5	The safety margin of less than 10 -4 should be explained so that there is a clear understanding of what this is measuring, what equivalent is demonstrated trough this value. It needs to be put un the overall context of demonstrating no risk of harm to the patient. Closed processing for example is a mitigation for not being able to demonstrate viral clearance.	
Lonza	1199		Annex 5	Annex 5 is focusing on monoclonal antibodies. However the document would benefit from recommendations on other modalities. For example, recommendations for AAVs would be extremely useful as for these, particles cannot be distinguished from the AAVs themselves (which particles should therefore be used in that instance?). AAVs do not contain endogenous retroviruses, for example, this should be made clear. Data on other modalities exist in the literature and should be used for a further appendix covering other modalities.	An approach to the level of clearance as a balance against substrate testing should be provided for modalities other than those referred to in revision 1 of Q5A.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Lonza	1199		Annex 5	Industry wide survey on what is currently is used in industry across the different modalities, with all demonstrating safety. Should there be any commentary here on how no such starting number may be available or appropriate in other modalities or if it is appropriates in some cases, then what examples might those be ?	For consistency of approach as other modalities are incorporated over and above traditional biologics.
BioPhorum	1199		Annex 5	Annex 5 is focusing on monoclonal antibodies. However industry would also very benefit from recommendations on other modalities. For example, recommendations for AAVs would be extremely useful as for these, particles can not be distinguished from the AAVs themselves (which particles should therefore be used in that instance?). AAVs do not contain endogenous retroviruses, for example, this should be made clear. Data on other modalities exist in nthe litterature and should be used for a new appendix covering other modalities.	
BioPhorum	1199		Annex 5	Industry wide survey on what is currently is used in industry accross the different modalities, with all demonstrating safety. Is a number needed for other cell lines s?	
Lonza	1203	1205	Annex 6	Platform validation approach and products from the same platform: The guideline does not give an idea of how much data is acceptable. How many products etc. or statistical approach. Is this definition or closer guidance something we want to set out in this revision ?	Can this document better define which process steps may lend themselves more easily to platform validation VC data. For example should chromatography approaches be discussed on a case by case basis prior to submission with the proposed strategy but inactivation (detergent and pH) and VRF, may more generally lend themselves to a platform approach.
Lonza	1203	1205	Annex 6	Prior knowledge: what are the expectations regarding prior knowledge, what are the references or in-house data approaches that will be accepted? Clarity is required on what kind of literature data are acceptable along with the proposed / accepted statistical approach.	Although a prescriptive approach cannot be given here, further guidance on what statistical approaches and the use of prior knowledge should be described. For example a company's prior knowledge of the same process capacity for VC with different products and the risk based approach and statistical analysis that goes with that described in outline.
BioPhorum	1203	1205	Annex 6	Platform validation apporach and products from the same platform: The guideline does not give an idea of how much data is acceptable: one product, 2 or 3? 4 to 6? What is the minimum data set that would be acceptable, as this is not harmonized accross EMA agencies for example.	
BioPhorum	1203	1205	Annex 6	Prior knowledge: what are the expectations regarding prior knowledge, what are the references that will be accepted? Clarity is required on what kind of litterature data are acceptable	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1209	1209	Annex 6: Viral Filtration	Major consensus topic: Document structure & consistency The use of the word 'validation' in this context seems somewhat loose. If prior knowledge derives from in-house experience, do these data need to be from qualified assays?Major consensus topic: Cocument structure	EFPIA suggests to rephrase the sentence to: “In this context, as opposed to product-specific process validation, platform validation….”
EFPIA	1211	1213	Annex 6	Major consensus topic: Prior knowledge It is unclear what " all the data avilable" means.	Recommend to change to "relevant platform data" instead of "all data"as is written in line 758
Lonza	1217	1217	Annex 6	Inactivation	Recommendation is therefore to remove all mentions of Triton X-100.
BioPhorum	1217	1217	Annex 6	Add high pH, both are used for viral inactivation (especially for new processes)	Recommendation is therefore to remove all mentions of Triton X-100.
BioPhorum	1219	1219	Annex 6	MuLV not XMULV, both types of MuLV are used - as reported in the table. Other retroviruses may also be included in the evaluation. Why is this specific virus called out here?	
EFPIA	1227	1227	annex 6	Major consensus topic: Prior knowledge Not only XMuLV clearance is being assessed in prior knowledge. For VF in table A-4 the parameters are for parvovirus (refer to title in line 1322) not XMuLV.	Recommend replace 'XMuLV' with 'virus'
PPTA	1243	1272	Annex 6	Triton X-100 may not be the best example to include given it's REACH regulation (Triton use was banned since January 2021)	Industries are working on Triton substitution. Suggest to use other example, i.e. Tween 80 (or PS80).
EFPIA	1249	1249	Annex 6	De-capitalize "monoclonal antibodies" for consistency with remainder of document.	minor editorial comment

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Lonza	1252	1254	Annex 6	There is a contradiction between the paragraph starting on line 1250 and the rest of the guideline: Triton X-100 is indeed on the European REACH list and is therefore not permitted for use in Europe. Other countries like the UK and Switzerland are also moving in this direction and others are likely to do so in the forthcoming years. Recommendation is therefore to remove all references of Triton X-100 as it has been banned under the environmental regulatory framework. Industry recognizes that at the moment there are not many alternatives. It is however expected that when they become available, new detergents will be defined with the same approach, for example through the definition of an ASTM standard but once a body of data is accumulated.	Recommendation is therefore to nuance wording to reflect the current state of industry and to future proof the document as these alternatives are not prescribed here but should be demonstrated fit for purpose with respect to VC, removability and safety. Reference to Triton X-100 in this document is again too specific and detergent inactivation and the implementation of new detergents and / or chemical agents could be referred too instead. Triton X-100 is subject to removal under Environmental regulation and not Biopharmaceutical regulation and so its discussion here is too specific and detergent s in general and a potential generic approach (e.g. via ASTM standard) could be referred to without specific detergent references.
BioPhorum	1252	1254	Annex 6	There is a contradiction between the paragraph starting on line 1250 and the rest of the guideline: Triton X-100 is indeed on the European REACH list and is therefore not permitted for use in Europe. Other countries like the UK and Switzerland are also moving in this direction and others are likely to do so in the forthcoming years. Recommendation is therefore to remove all mentions of Triton X-100 as it has been banned from the environmental regulatory framework. Industry recognizes that at the moment there are not many alternatives. It is however expected that whenthey become available, new detergents will be defined with the same approach, for example through the definition of an ASTM standard.	Recommendation is therefore to nuance wording to reflect the current state of industry
Charles River Laboratories	1264	1265	Annex 6	The evaluation made in tables A-2 to A-4 and corresponding text address MuLV (or retroviruses) specifically as outlined in line 1219-1220. "MuLV" should be added to the headline of table A-2 to further emphasize the relevance for MULV (or retroviruses); similar to table A-3	Adding "MuLV" in the Headline to the table A-2
Charles River Laboratories	1266	1270	Annex 6	The conclusion made in these lines are unclear with respect to application. Question: can Annex 6 and the comclusios made here be referenced to justify e.g. elimination or reduction of SD/Triton MuLV clearance experiments when a production process meets the outlined conditions (e.g. 1% polysorbate 80 and 0.3%TNBP for ≥6h at ≥23°C) without justification through prior knowledge data (external and in house data)? Or is this just an example an cannot be referenced and must be justified by external/in house data?	
EFPIA	1266	1269	Annex 6	Major consensus topic: Prior knowledge Based on this line, it appears that "SD," especially in the table, refers to this specific combination. This should be clarified.	Add "as examples": "Thus, as examples, consistent with....." in line 1266

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
ViruSure GmbH- Andy Bailey	1274	1280	Table A-2	Protein precipitation is an important factor for any inactivation step	As with detergent treatment, the presence of precipitates can protect virus from inactivation at low pH. Proteins therefore that have a tendency to precipitate at low pH can sometimes impact on the robustness of the process, with low levels of residual virus still detectable after treatment. This should be included as a possible critical factor for consideration
Albrecht Gröner	1274	1275	Annex 6	"Low pH treatment inactivates enveloped viruses by denaturing proteins located in the viral envelope, thus disrupting the lipid envelope." Denaturing proteins located in te viral envelope results in preventing the adsorption of viruses at the cell membraned and, thus, the infection of the cell but the lipid envelope is not disrupted as shown in Brorson et al. Bracketed generic inactivation of rodent retroviruses by low pH treatment in monoclonal antibodies and recombinant proteins. Biotechnol Bioeng 2003;82:321-29: "However, ultracentrifugation over sucrose density gradients did not reveal density changes in X-MLV following low-pH treatment. The buoyant density of XMLV before and after low-pH treatment was 1.17–1.18 g/mL (data not shown)."	Low pH treatment inactivates enveloped viruses by denaturing proteins located in the viral envelope, thus preventing the adsorption to and infection of cells.
Charles River Laboratories	1286	1288	Annex 6	siehe comment above - can this conclusion be used to justify elimination/reduction of low pH experiments if the specified conditions are met or must this still be documented via external/in house data?	
Lonza	1291		Annex	Title Virus Filtration should read 'Virus Reduction Filtration'	more accurate
Charles River Laboratories	1291	1291	Annex 6	This chapter is not clear if the focus is on MuLV (as oulined in lines 1219-1220 and lines 1300-1301) or large enveloped viruses in general. Subsequent text includes prior knowledge usage for large enveloped viruses generally and even parvoviruses (lines 1317-1319). Considering the current practise of MAA/BLA viral clearance studies and virus filtration steps a more clear statement would be desired. Can a parvovirus only be used (worst case model) if justified by prior knowledge (external and in house data) or should a second small virus be included for robustness demonstration?	
Pall Life Sciences	1292	1292	Annex 6	"The mechanism of action of virus filtration is size-based particle removal" Add "Primary" to acknowedge that some other mechanisms may be involved	The primary mechanism of action of virus filtration is size-based particle removal
Pall Life Sciences	1292	1295	Annex 6	This misses flow decay as a critical parameter. Flow decay is a published and well accepted risk to virus retention and should be included.	Add to text and add to Table A-4. Recommended table text includes reference to flow decay caused by pore blockage and that other types of fouling (e.g. surface fouling, cake formation) will not necessarrily be a risk.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1297	1299	Annex 6	Major consensus topic: Prior knowledge Original text: “However, in cases where the virus particle size and pore size is similar the influence of the potential interaction on flow dynamics and virus retention is not fully understood” It is unclear what the influence of potential interaction relates to and how that affects virus retention. If the protein is interacting with the virus, it will only make the virus larger and easier to remove by a filter.	Lines 1297 - 1299 seem to address potential virus breakthrough caused by e.g. pressure interruptions. This is not related to protein-virus interaction, and seems misunderstandable. Suggest to ask for clarification how the statement in line 1298-1299 is related to protein-virus interaction.
Pall Life Sciences	1300	1300	Annex 6	"This section focuses on using prior knowledge and in-house experience in virus filtration of other products to claim retrovirus removal by small and large virus-retentive filters." There is no section and the previous two paragraphs do not form part of this focus.	Create new section title or change to "The rest of this section…". See comments on Table A-4 which is not related to this section focus, so should be referenced after the first two virus filtration paragraphs.
Pall Life Sciences	1301	1301	Annex 6	small and large virus-retentive filters. Re-word to indicate filters that remove small and large sized viruses	Filters that remove small and large sized viruses
Asahi Kasei Bioprocess Europe S.A./N.V.	1302	1305	Annex6	Factors that impact efficient retrovirus removal by small-virus filters are well understood with respect to variation of process parameters such as membrane type, flow- or pressure-controlled filtration mode, and pressure interruptions. Based on predictability and robustness of virus removal this process step is considered suitable for a platform validation approach.	Factors that impact efficient parvovirus removal by small-virus filters are well understood with respect to variation of process parameters such as membrane type, flow- or pressure-controlled filtration mode, and pressure interruptions listed in Table A-4. Based on predictability and robustness of virus removal this process step is considered suitable for a platform validation approach when applying large retrovirus removal with small virus removal filters. Reason: Table A-4 is a summary of process parameters and their potential impact for parvovirus clearance with small virus removal filters as well, but not for large retroviruses. We also suggest revision the description to make more explicit the cases where platform validation can be applied
CSL Behring	1306	1307	Annex 6	Given that virus filtration is based on a size-exlusion mechanism, is the use of parvoviruses as a worst case model considered sufficient when validating the virus retention capacity of small pore virus filters? Specifically, in cases where complete retention of parvoviruses is demonstrated, are addiotional validation studies still required to demonstrate retention of viruses of larger size?	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Pall Life Sciences	1306	1306	Annex 6	"For virus removal using small virus filters…". Indicate the viruses are small in size, not the filters	For virus removal of small sized viruses
Lonza	1306	1306	Annex 6	Use of misleading terminology "small virus filters" - this could be read as "small scale filters", it is small pore size filter	propose using the term in line 1314 "small-virus retentive filter"
Charles River Laboratories	1306	1312	Annex 6	In case one uses reduction factors of parvovirus as surrogate number for retrovirus but minimal residual infectivity was found (the parvovirus LRF will miss the ≥ symbol and the LRF will be considered the "real" reduction and not greater than) what does this mean for the deduced theoretical LRF for retroviruses? Can one still reasonably assume no residual infectivity for MuLV and claim the same factor but with the symbol ≥ for greater than?	
BioPhorum	1306	1306	Annex 6	Use of misleading terminology "small virus filters" - this could be read as "small scale filters", it is small pore size filter	propose using the term in line 1314 "small-virus retentive filter"
Biosimilar Medicines Group - Medicines for Europe	1306	1307	Annex 6	The guideline allows for the companies to use their in-house data from parvovirus and retrovirus removal to build a platform retrovirus clearance claim for commonly used small virus filters. Further elaboration or examples of such approaches would be helpful to include in the Annex. For example, the possibility of selecting a representative virus suitable for the purpose of each step/filter (different virus for different steps, i.e. parvovirus for virus filtration and retrovirus for other steps) to determine the safety margin for viral clearance could be discussed in the guideline with appropriate examples.	
Lonza	1315	1316	Annex 6	A thorough understanding of the impact of pressure interruption': please clarify if there is an expectation that robustness testing will be completed to assess the impact of unexpected interruption in flow or that data will be provided that assess the impact of routine process interruptions. Recommendation of the following paper as an example to aid the discussion: https://bioprocessintl.com/downstream-processing/viral-clearance/worst-case-conditions-for-viral-clearance/	Although specific references may not be included in this document a reference to the use of what industry considers to be worst case conditions may be appropriate. For VRF the concept of pressure perturbations / interruptions low pressure and in the context of multiple events should be considered in the small scale model as a representation of the situation in the full scale / at scale process.
Lonza	1315	1316	Annex 6	EMEA 398498 / 2009 document does not state EMA expectations with regard to process interruptions for investigational medicinal products. This guideline and the EMA document need to be aligned ideally although it is acknowledged that the purpose of this edit is not to align regional / local documents but acknowledge where the industry has shifted in approach / knowledge.	Although the EMA document would not be referenced here, this document could refer to the industry concern over the passage of small viruses across virus retentive filters and how the conditions of pressure, flow and flow perturbations influence this phenomenon. Demonstration should be via the scale down model as a reflection of the at scale / full scale manufacturing process.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1315	1316	annex 6	Major consensus topic: Prior knowledge Align description of critical VF parameters to earlier reference in lines 1292 - 1295. Need to clarify why GMP is mentioned here and what "conserved" mean.	Replace line 1315-1316 with "A thorough understanding of volumetric throughput of product intermediate and flush, pressure including process pause reflecting manufacturing conditions should be maintained."
BioPhorum	1315	1316	Annex 6	A thorough understanding of the impact of pressure interruption': please clarify if there is an expectation that robustness testing will be completed to assess the impact of unexpected interruption in flow or that data will be provided that assess the impact of routine process interruptions. The team also recommends the following paper to aid the discussion: https://bioprocessintl.com/downstream-processing/viral-clearance/worst-case-conditions-for-viral-clearance/	
BioPhorum	1315	1316	Annex 6	EMEA 398498 / 2009 document states the EMA expectations with regards to process interruptions for investigative medicines. This guideline and the EMA document need to be aligned.	
EFPIA	1317	1321	annex 6	Major consensus topic: Prior knowledge/Confirmatory validation run for virus filtration This requirement may change with evolving process understanding. In some EFPIA member companies, the confirmatory run for virus filtration precluded using prior knowledge in the past. The benefit and the approach to build an LRV claim for the new product seem unclear.	EFPIA suggests to a) add "unless justified" at the end of the sentence, and b) re-phrase lines 1320-1321 by "The type of virus filter is important for virus reduction and its robustness with respect to impact of process parameters and may be considered when designing platform data.” In addition, EFPIA would like to ask EWG a) for a rationale for the confirmatory run and b) which data are required to support platform validation without a single confirmatory run for virus filtration.
Albrecht Gröner	1317	1319	Annex 6	"If using prior knowledge and in-house experience from other products to claim parvovirus removal, at least one confirmatory product-specific validation run using a parvovirus should be performed" is strongly supported	If using prior knowledge and in-house experience from other products to claim parvovirus removal, at least one confirmatory product-specific validation run using a parvovirus should be performed under worst case conditions as high volumetric filter load, low pressure and flow interruption
Alliance for Regenerative Medicine	1317	1319	Table A-3	Lines 1317-1319 fall in the Annex on use of prior knowledge and platform validation approaches. Perhaps this relates to concerns regarding platform validation for some steps and therefore states the requirement for a “confirmatory product-specific validation run” for virus filtration - this seems contradictory to the platform validation concept.	Suggest to provide clarification as to expectations for analytical validation where a defined platform is used for manufacture - to maximize platform data usage.
Lonza	1320			Virus filter should be virus reduction filter	modify wording to read 'virus reduction filtration'

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
ViruSure GmbH- Andy Bailey	1322	1324	Table A-4	Data exists to support a minor impact of pH on virus breakthrough for some matrices	pH ia described as having no negative impact on virus clearance due to size based removal. Some studies though have demonstrated an impact of pH on virus breakthrough. It is therefore suggested to change "No negative impact" to "Limited negative impact"
Pall Life Sciences	1322	1324	Annex 6	Table A-4 is not referenced in the text.	Add similar sentence to tables A-1 to A-3 and A-5 in the text, before the section talking exclusively about larger sized viruses using prior knowledge and parvovirus data for validation.
Lonza	1322	1323	Annex 6	Title of table is slightly misleading	Change wording to read '..by Small Pore Virus Retentive Filters'.
EFPIA	1322	1324	Annex 6	Minor editorial, Table A-4, Row 1; suggestion for further clarity	"low level parvovirus passage has been observed (with increasing throughput) depending…..
EFPIA	1322	1324	Annex 6	Minor editorial, Table A-4, Row 2; suggestion for further clarity	Low level parvovirus passage has been observed (depending on filter type/brand)
BioPhorum	1322	1322	Annex 6	Studies introducing parvovirus are also typically performed, however this is not part of this guideline	
PPTA	1322	1323	Table A-4	Table A-4 states volumetric throughput as high impact "Volumetric throughput of product intermediate loaded on the virus filter" – experience has shown that this may rather be high protein throughput.	Proposal to also consider protein throughput, by changing the wording to "*Volumetric/ protein throughput of product intermediate loaded on the virus filter*"
PPTA	1322	1323	Table A-4	Table A-4, Line "*Pressure": "Pressure should not exceed the upper limit for filter operation. …*"	Proposal to change to "*Pressure should significantly not exceed the upper limit for filter operation. …*"
Pall Life Sciences	1323	1323	Annex 6	"Small virus-retentive filters". Re-word to indicate the viruses are designed to remove small sized viruses	
Pall Life Sciences	1323	1323	Annex 6	"Low pressure can be worse case for a specific membrane type." This implies that there are some filters or specific subsections of membrane designs where low pressure is not a worse case, which is not true. The level to which the pressure has to drop is very different between individual membranes and there appear trends in specific mebrane characteristics. The word can is also inapproriate with with a lack of data on high pressure penetration of virus and a large body of data and publications on low pressure and pressure interruption effects.	Low pressure is usually worse case but should be based on risk assessment including prior knowledge of the level of impact on the selected membrane type.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Pall Life Sciences	1323	1323	Annex 6	Table A-4, Table A-2, Table A-3. Suggest adding a footnote regarding the "Potential Impact" to indicate that this should be determined by the end user based on risk assessment, prior knowledge assessments etc	
Lonza	1323	1323	Annex 6	Pressure: wording could be made clearer: high and low pressures are important as well as pressure interruptions. However pressure fluctuations that do take place during normal commercial manufacture should also be addressed. The small scale models that are typically used need to represent all these scenarios. Small scale studies typically include stops, increased pressures (above and beyond typical pressures), lower pressures, fluctuations and stoppages. Small viruses combined with multiple pressure interruptions are a worst case scenario.	Low pressure as the 'worst case condition) encouraging / exacerbating virus migration through the membrane under low pressure should be referred to in this table.
Lonza	1323	1323	Annex 6	pH and ionic strength of the buffer can also have an impact on parvovirus retention where there is pressure interruption. This is currently not captured in the table. Please add a footnote on interactions, combinations at extremes can provide worst case conditions: for example, high ionic strength, high pH and pressure drop for example.	This could be added as a footnote to the table where a process has extremes of ionic strength or conditions that may be a concern for exacerbating small virus passage across VRFs, then these parameters should be considered in such cases. Based on the industry wide survey, 2022, these parameters would not generally be considered under 'normal' operating parameters but have been demonstrated to influence breakthrough.
EFPIA	1323	1323	annex 6	throughput parameters are high impact yet low level parvovirus passage is referenced. Recommend to remove 'low level' since virus filters are not expected to be absolutely retentive and a low level of passage does not significantly affect the overall LRV of the step.	recommend to align throughpbut rationale to pressure rationale. Load and Buffer throughput rows rationale should read: "high throughput can be worst-case for specific membrane types"
BioPhorum	1323	1323	Annex 6	Pressure: wording could be made clearer: high and low pressures are important as well as pressure interruptions. However pressure fluctuations that do take place during normal commercial manufacture should also be addressed. The small scale models that are typically used need to represent all these scenarios. Small scale studies typically include stops, increased pressures (above and beyond typical pressures), lower pressures, fluctuations and stoppages. Small viruses combined with multiple pressure interruptions are a worst case scenario.	
BioPhorum	1323	1323	Annex 6	pH and ionic strength of the buffer can also have an impact on parvovirus retention where there is pressure interruption. This is currently not captured in the table. Please add a footnote on interactions, combinations at extremes can provide worst case conditions: for example, high ionic strength, high pH and pressure drop for example.	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
BioPhorum	1323	1323	Annex 6	Table A-4, Table A-2, Table A-3. Suggest adding a footnote regarding the "Potential Impact" to indicate that this should be determined by the end user based on risk assessment, prior knowledge etc	
PPTA	1323	1323		Minor comment, but there are different uses of 'High' and 'high' in the table	Align use of 'High' and 'high' in the table
EFPIA	1325	1418	Annex 7	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 MAJOR: A section regarding the steps where tests should be performed would be of value between section 7.1 Introduction and section 7.2 Testing for viruses. In addition, in Table A-5, the column about the cell substrate (MCB, WCB, cells at the LIVCA) could be removed and only a cross-reference made in the narrative, as suggested in the next column.	Add a section "Manufacturing steps for viral vector production" This section should cover: 1-Cell substrates with a cross-reference to section 3, and the specific point of replication competent viruses. 2-The virus seeds (MVS, WVS) 3-The unprocessed bulk (or virus harvest) 4-The control cells
EFPIA	1325	1418	Annex 7	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 MAJOR: A new organization of the Annex is proposed for more consistency with the main guideline and for better lisibility. The multiplicity of footnotes make this Table difficult to understand. It is recommended to keep only the necessary footnotes, as footnote, and consider other parts in the narrative of the annex, as suggested. Typically, the explanations about the tests should be in the narrative (as it was done for cell substrate in the main part of the guideline), and the footnotes for the rationale to do a test at a specific step could be kept as footnotes. Explanation about how the control cell should be tested should be added.	See the attached file of proposed for Annex 7, section 7.2 and Table A-5 [to help address the repeat queries for more clarity in table 1, such as the meaning of "+", (+) and to help consolidate the increasing # footnotes, EFPIA propose an suggested alternative table A-5 layout in a separate attached file (word doc) and the transfer of the narrative from the footnotes to Section Annex 7.2. Within the table, the testing requirements are provided alongside where risk based cosiderations apply, and the assoiciated footnote retained, but as a cross reference to the relevant narrative sections associated with the test. in addition, the cell line qualification aspect described for RCV could be transferred to Table 1]. As footnote g is critical to help decision tree for in vivo testing, request to ensure the full content from footnote g is transferred to Section 3.2.3 if EWG agree to a proposed alternative layout for Table 1].

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1325	1327	Annex 7	Major Consensus Topic: Product Scope Why can this not just clearly spell out that only gene therapies/viral vectors are in scope and that other types of ATMPs and also cell therapies are out of scope. If that would have been called out in the introduction already (as stated before) that would be a lot more clear. But note that this annex then completely avoids the issue that there is still an expectation in some markets which consider cell therapies a GMO to make some claims around reduction factors. Would some of the statements on assay limitations, estimation of sample size/ability to dected virus etc not be more generally applicable?	Update the scope statement in the introduction and here to make it more clear what's in and out of scope.
ProPharma Group <Erik Schagen & Kristiena Abbink>	1325	1446	Annex 7	One of the objectives of the revised guideline is to reflect on challenges provided by new classes of biotechnology products. In the manufacturing of a significant part of these biotechnology products an MVS and WVS is used. In the new guideline qualification of the MVS and WVS is included in an Annex at the end of the document. In view of the objective of the revision and the significant use of MVS and WVS, one could consider to present this information in a more prominent and profound way. It is questioned why not a separate chapter/section is dedicated to the qualification of MVS and WVS instead of an Annex.	As indicated, we recommend to include a separate and detailed Chapter on virus seed qualification. In view of the current structure of the guideline and Annex 7, which provides a more general overview regarding viral safety of viral vector (derived) products, it is acknowledged that this will be quite a challenge. As temporary fix, it is suggested to insert the Annex 7 as Chapter 7: Viral safety of biotechnology products using cell banks and virus seeds. This will increase it's visibility. A more in depth discussion about virus seed qualification could be the objective of a future revision of the guideline.
Pall Life Sciences	1333	1334	Annex 7	"These products include Virus-Like Particles (VLPs) and protein subunits that are produced using baculovirus/insect cells". There are multiple other expression systems used for such products (https://jnanobiotechnology.biomedcentral.com/articles/10.1186/s12951-021-00806-7). The section needs to be clear that these are being included based on the nature of the product or the baculovirus in the expression system or both. Same comments apply to Line 18, Section 1.	"These products invlude Virus-Like Particles (VLPs) and viral protein subunits produced in a variety of expression systems." or "These products include various therapies produced using baculovirus vectors in insect cells."
Charles River Laboratories	1334	1335	Annex 7		".....nanoparticle based vaacines and therapeutics, and viral vector products such as AAV.
Pall Life Sciences	1337	1341	Annex 7	AAV is no longer commonly produced using a helper virus, but via triple transfection or engineered stable producer cell lines. Helper viruses enable virus and not protein expression. There is no need for this distinction here. There are no products that are helper virus dependent specifically and the distinction is confused by the lack of clarity on the baculovirus as a helper virus.	Remove whole paragraph.
BioPhorum	1338	1339	Annex 7	Add recombinant adeno-associated viruses in the bracket	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
BioPhorum	1339	1341	Annex 7		Generally for AAV gene therapies, a virus isn't used to bring the helper protein needed to generate the recombinant AAV particles (helper function is brought by a helper plasmid that is co-transfected into the production cells with other plasmids for the gene of interest and packaging elements); in this case such recombinant AAVs do not rely on a helper virus to enable expression and are in the helper-virus independent case (transient transfection case). Current wording does not reflect the current or future state of industry: None of the AAVs on the market use helper-viruses and none of our organizations are intending to use them either. To future proof the document, please remove specific references and keep the discussion more general
EFPIA	1348	1348	Annex 7	Major Consensus Topic: Helper Virus Description/Defintion Clarification is requested on expectations for helper viruses, ex. baculovirus. Wouldn't baculovirus be considered a process-related viral contaminant as well?	Recommend to revise line 1348 accordingly to clarify expectations. Two potential options for revision exist: (1) "Furthermore, helper viruses and expression system-associated viruses used for production are considered process-related viral contaminants", or (2) "Furthermore, helper viruses and protein-expression virus vectors used for production are considered process-related viral contaminants"
EFPIA	1350	1352	Annex 7	Major Consensus Topic: Helper Virus Description/Defintion Clear guidance is provided on adventitious viruses and helper viruses. Expectations for a third category, protein expression viruses, is unclear. Hence, clarification is requested on the categorization and expectation for clearance of protein expression viruses. For example, one could consider a helper independent baculovirus to fall under "protein-expression virus vectors" as defined in line 1338.	Proposal is to revise text in lines 1350-1353 to include protein expression virus vectors. Revised text as follows "Viral safety and contamination controls of new product types should be assured through the application of a comprehensive program of material sourcing, virus testing at appropriate steps of manufacture and removal and/or inactivation of adventitious viruses, helper viruses, and protein-expression virus vectors by the manufacturing process"
EFPIA	1350	1354	Annex 7	Minor Shared Theme: Expand on The Three Principles & Incorporate Risk Assessment Language Section 7.1: Consider including the use of facility controls as well (clean equipment, air, closed systems, etc.)	See column F

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Pall Life Sciences	1354	1354	Annex 7	Many processes are being developed with virus removal applied to raw materials, not just media, where there is limited clearance.	"If process virus clearance is limited, virus safety should focus on the testing and control of the raw materials and reagents and the manufacturing process. In addition virus clearance steps such as filtration can be applied to the raw materials entering the process to limit the risk of adventitious infection."
EFPIA	1367	1371	Annex 7	Minor: Recommend to consider development of a companion document that provides additional details/proposals w.r.t. the risk assessment described in this section to accompany ICHQ5AR2. For example, some information that describes the number of serum free passages that would be necessary for a certain cell bank to be designated as "serum free" cell line	
EFPIA	1370	1371	Annex 7	Major Consensus Topic: Helper Virus Description/Defintion Clarification is requested on expectations for protein-expression virus vectors w.r.t control strategy. Recommend to revise the sentence to include protein-expression virus vectors accordingly.	Proposed revised text as follows "vector, the raw materials and reagents and culture methods used, the reliance on helper virus(es) and/or protein-expression virus vector(s), and the capacity of the manufacturing process to inactivate and/or remove viruses."
Pall Life Sciences	1372	1372	Annex 7	Very confusing footnotes.	Recommend revising this table significantly and include information from the footnotes in the body of the text or the table itself where appropriate. Need to avoid the use of "l" especially for the most common footnote used in empty cells where it appears to be a "1". If keeping the table in it's current state, at least re-assign the footnotes in a logical alphabetical order.
EFPIA	1372	1418	Annex 7	Major: About the Antibody production assays: these tests are specific to rodent contaminants, and to the use of rodent cell line, or raw material that could have been contaminated by rodents. It should be explain.	Suggest to add that Antibody Production Assays are specific to rodent viral contaminants, and could be added according to the risk assessment.

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1372	1380	Annex 7	MAJOR: Virus seeds and UPB must be tested for In Vitro or NGS. Superscript "a" states "if viral vector or viral derived product cannot be neutralized a validated alternative assay can be used". Superscript "b" states "NGS should be considered to replace In vivo and may be used to supplement or replace in vitro, based on assay suitability and risk assessment". Since the door is open for NGS or alternative options is it imperitive that the criteria to substitute In Vitro be "Cannot be neutralized"? Can we request that alternative assays can be utilized regardless of whether neutralization is possible or not? Considering changing ethics in the EU it may not be long before generating anitisera is no longer an option. If NGS can replace In vitro as a general rule of thumb can we ammend the statement for superscript "a"? Also note the option to replace in vivo or in vitro without any pre-requirements on the ability to neutralize or not if provided in Table 1, superscript "j" on line 1014. Section 3.2.5.2 supports the replacement of In Vitro AVA with NGS specifically for process matrices where "there is assay interference as a lack of effective neutralization" (line 261). So again the "cannot be neutralized" is not a definitive requirement and limited effectiveness can be a rational for alternative testing options.	Replace in superscript "a" line 1376, "If viral vectors and viral vector-derived products cannot be neutralized, a validated alternative can be used. By "If viral vectors and viral vector-derived products cannot be neutralized or if neutralization has limited effectiveness impacting the performance and sensitivity of the assay, then a validated alternative can be used.
EFPIA	1372	1418	Annex 7	Editorial: Virus and viruses are used interchangeably for virus in plural. Consider harmonizing terms throughout the guideline. IN GENERAL, there are some repetitive messages that could be streamlined for clarity.	
EFPIA	1372	1372	Annex 7	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Section 7.2: Table A-5: Explain the pluses and minuses in the Table in the main body of the text. Specifically, explain the difference between "+" and "(+)". Minimize the length of the footnote in favor to an explanation prior to the Table on how to interpret the Table based on the symbols.	See column F
EFPIA	1372	1372	Annex 7	Major Consensus Topic: Replication competent virus testing In table A-5, for 'replication competent viruses', it is indicated that the test should be conducted for cells (i.e. MCB, WCB, Cells at the LIVCA), Virus seed, Unprocessed Bulk, and Drug Substance. However, performing such a test is not logical for cell banks for adenovirus-vectored vaccines since they contain no vector. Testing for replication competent viruses is also not necessarily applicable in all cases for Unprocessed Bulk and Drug Substance, specifically when the risk of the vector acquiring or re-acquiring replication competency has been shown to be negligible in a risk assessment.	For cells (i.e. MCB, WCB, Cells at the LIVCA), change the "+" to "+ l". Adding the reference to footnote 'l' is to reflect that testing should be performed based on a risk assessment. Similarly, the reference to footnote 'l' should also be added to in the same line to Unprocessed Bulk, and Drug Substance. [Additionally, EFPIA proposal to add footnote (or within table itself if adopting new proposed tables) that RCV testing of cell banks is only applicable for stably-transduced cell lines]

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
BioPhorum	1372	1372	Annex 7	Good information but confusing in its presentation. Not easy reading. Some VERY important considerations are captured in the footnotes, these should be clarified in the main body of the text. Safety margin acceptability is an example, the footnote status does not reflect importance for industry. A lot of exceptions are addressed. This format does not give clear instruction for each specific product, this is consistent with the format of Table 1 but still confusing. Consider repeating the table for specific situatios. It will make the document longer but easier to read and apply.	
BioPhorum	1372	1372	Annex 7	Is testing to be repeated for virus seeds when it has been performed on the working cell banks? Footnote k explains alternative approaches. This is an example of further exposure needed for a very important topic.	
Alliance for Regenerative Medicine	1372	1373	Annex 7, Table A-5		For clarity suggest not to include "or specific molecular assay" below and instead state here "virus specific assays" (removing "other").
Alliance for Regenerative Medicine	1372	1373	Annex 7, Table A-5	The stated definition of an Endogenous Virus is "Viral entity whose genome is part of the germ line of the species of origin of the cell line and is covalently integrated into the genome of animal from which the parental cell line was derived. In this guideline, intentionally introduced, non-integrated viruses such as Epstein-Barr Virus used to immortalise cell substrates or Bovine Papilloma Virus." Retroviruses are indicated as endogenous. Adventitious contamination with retroviral vectors could occur in a facility. These would typically have reverse transcriptase. Suggest not to use the terminology "endogenous" to divide tests in this table.	Suggest to list virus types/tests and remove "Test for adventitious or endogenous viruses" and "Tests for Endogenous, Helper and Replication Competent Viruses, as applicable" and instead list: - in vitro assays or NGS - in vivo assays or NGS - virus-specific tests - antibody production assays - retroviruses - residual helper viruses - vector-derived replication competent viruses
Alliance for Regenerative Medicine	1372	1373	Annex 7, Table A-5	The stated definition of an Endogenous Virus is "Viral entity whose genome is part of the germ line of the species of origin of the cell line and is covalently integrated into the genome of animal from which the parental cell line was derived. In this guideline, intentionally introduced, non-integrated viruses such as Epstein-Barr Virus used to immortalise cell substrates or Bovine Papilloma Virus." Retroviruses are indicated as endogenous. Adventitious contamination with retroviral vectors could occur in a facility. These would typically have reverse transcriptase. Suggest not to use the terminology "endogenous" to divide tests in this table.	Suggest to list virus types/tests and remove "Test for adventitious or endogenous viruses" and "Tests for Endogenous, Helper and Replication Competent Viruses, as applicable" and instead list: - in vitro assays or NGS - in vivo assays or NGS - virus-specific tests - antibody production assays - retroviruses - residual helper viruses - vector-derived replication competent viruses

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1373	1390	Annex 7	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Annex 7, Section 7.2: Major: Footnotes of table A-5: some footnotes apply to table 1 when considering cell testing. Placed in section 7.2 make them applicable for viral vectors and viral vector-derived products only, in particular footnote e.	Recommend to insert footnote a, b, c, d, e, i, in table 1 or in the respective sections e.g., - section 3.2.1 and/or section 4 for footnote e - section 3.2.2 for footnote a - section 3.2.4 for footnote d - section 3.2.5 for footnotes b and c - section 3.2 for footnote i
EFPIA	1373	1374	Annex 7	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing The requirement for secondary passage, should only be a requirement for master cell bank testing. Secondary passage testing of virus seed and bulk might by required based on risk assessment. It should not be a requirement for WCB testing, if the MCB was tested using a secondary passage strategy.	The indicator cells cultures should be observed for at least 2 weeks. A further secondary passage of 2 weeks of observation should be performed based on risk assessment.
EFPIA	1373	1374	Annex 7	Major Consensus Topic: IVV Assay Durations for the Various Stages in Manufacturing Major: Is the second 2 weeks always necessary? Are there guardrails if it isn't? The 2 weeks vs. 4 weeks in vitro test should be based on risk assessment. eg. for testing of harvest, it's 2 week is there are no animal/human raw materials used.	aTesting should be performed on permissive cell lines, based on risk assessment. The indicator cells cultures should be observed for at least 2 weeks, with a further secondary passage of 2 weeks of observation, if needed.
EFPIA	1373	1373	Annex 7	Minor: Footnote b: request to clarify the end of the first line"based on risk assessment". This suggests this testing can be RA based, though first row of table suggests this is required. Recommend to clarify/align the goals accordingly.	
EFPIA	1373	1375	Annex 7	Minor: Recommend to add CPE as read out	
PPTA	1373	1374	Table A-5	Footnote a) …"The indicator cells cultures should be observed for at least 2 weeks, with a further secondary passage of 2 weeks of observation" Requirement not consistent with Section 4, Testing for viruses in unprocessed bulk, where the requirement is in Line #321 to 322 "... the indicator cell cultures should be observed for at least 2 weeks."	To be consistent with Section 4, Line 322, clarification to "Footnote a" should be added that for testing for viruses in unprocessed bulk, based on a risk assessment, observation of indicator cells for at least 2 weeks is sufficient.
Alliance for Regenerative Medicine	1373	1373	Annex 7	Orthography	change from "The indicator cells cultures..." to "The indicator cell cultures..."

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Alliance for Regenerative Medicine	1373	1373	Annex 7	orthography	change from "The indicator cells cultures..." to "The indicator cell cultures..."
PPTA	1374	1375	Table A-5	Footnote a) …"Include testing for haemadsorbing and hemagglutinating viruses"….	To be consistent with Section 3.2.2, Lines 206 to 207 ("*...followed by observation for both cytopathogenic and hemadsorbing/hemagglutinating viruses*"), proposal to change to "*Include testing for haemadsorbing or hemagglutinating viruses*"
EFPIA	1376	1406	Annex 7	The term arbovirus is used. This term is an informal description based on phenotype (transmitted by insects) of virus from very diverse families. Most members of this informal group belong the the family flaviviridae.	Avoid informal terminology - use the term flaviviridae instead.
EFPIA	1377	1378	Annex 7	Section 7.2: Footnote:"Testing should be performed on the virus seed and the unprocessed bulk harvest before downstream processing." Sentence is too restrictive. Consider changing to "Testing should be REPORTED FOR the virus seed and the unprocessed bulk harvest before DRUG SUBSTANCE IS RELEASED."	Consider changing to "Testing should be REPORTED FOR the virus seed and the unprocessed bulk harvest before DRUG SUBSTANCE IS RELEASED." [Alternatively, if the meaning is to ensure AVA testing is performed on the samples which had not received treatment/ purification steps to reflect worst case detectability, could the current sentence further clarity this meaning - examples per Section 4 " ...samples removed for testing before further processing represent one of the most suitable levels at which the possibility of adventitious virus contamination can be determinnd with a high probability of detection"].

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1379	1379	Annex 7	Major Consensus Topic: Alternative Layout for Testing Tables 1 and A-5 Annex 7: Table A-5: Major: Alignement between Table 1 and Table 1-5 footnotes footnote b - "When applicable, broad NGS should be considered to replace the in vivo adventitious virus tests and may be used to supplement or replace the in vitro tests based on assay suitability and risk assessment." This sentence should be also used for Table 1 and related core text. General comments: Table 1 and Table A-5 footnotes are not harmonized - eg: targeted molecular or Trageted NGS, both for MAP/HAP/RAP	Proposal to combine Table 1 footnote j (line 1014-1015) "When applicable, NGS should be considered to replace the in vivo test and may be used to supplement or replace the in vitro and other virus specific tests based on assay suitability and risk assessment" and Table A-5 foonote b (line 1379-1380) "When applicable, broad NGS should be considered to replace the in vivo adventitious virus tests and may be used to supplement or replace the in vitro tests based on assay suitability and risk assessment." Resulting in a consensus for both footnotes: "When applicable, broad molecular methods (e.g. agnostic NGS) should be considered to replace the in vivo adventitious virus tests and may be used to supplement or replace the in vitro and other virus specific tests based on assay suitability and risk assessment" Alignment for MAP/HAP/RAP: Table 1: Antibody production tests or specific molecular assay Table A-5: Antibody production assays or specific molecular assay Proposed consensus for both tables: Antibody production assays or specific molecular assays Table 1: Virus specific PCR or targeted molecular methods Table A-5: virus specific NAT or targeted NGS Proposed consensus for both tables and line 230: Virus specific PCR or targeted molecular methods (eg. targeted NGS)
EFPIA	1381	1383	Annex 7	Minor: Recommend to be more inclusive in the type of cell lines referenced in this section. For example, consider also Vero or other cell lines?	
Charles River Laboratories	1384	1385	Annex 7	footnote d, see also related row in table A-5: The MAP/HAP/RAP assay is suitable for rodent derived production systems (see chapter 3.2.4). If non rodent cells are used this assay has limited value. We don't see a reason to have this assay specifically outlined in the table. The MAP/HAP/RAP assay is a species specific assay and falls under "other virus specific assays" in the table and the footnote c.	Removing footnote d and related row in table A-5 and adding MAP/HAP/RAP as a specific assay for rodetn based production systems under footnote c with the option to use alternative molecular based assay (NAT, targeted NGS)
EFPIA	1385		Annex 7	minor: Recommend to change text to read “and/or” reagents	“and/or” reagents

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1386	1390	Annex 7	Major: The retrovirus testing using RT assays at the MCB and virus seed is understood. However, the guideline also specifies RT testing on the unprocessed bulks. While based on risk assessment, can the guideline clarify as to when and why testing for RT activity at harvest stage would be necessary, as the RT assays can provide variable results, and as the products in scope are all subject to downstream virus clearance for retrovirus particles when retroviruses are detected at the MCB or virus seed.	Propose to elaborate as the risk-based reasons for RT testing at the unprocessed bulk [Alternatively, EFPIA propose to amend the wording from "should be performed..." to "may be performed based on risk assessment"]
EFPIA	1386	1389	Annex 7	Define “campaign”, see comment for lines 430 - 432, Section 6	
EFPIA	1386		Annex 7	minor: Recommend to add "retrovirus-like particles" to this sentence after retrovirus	
EFPIA	1389	1390	Annex 7	Please clarify if the intention is for retrovirus testing is required on every unprocessed bulk or is tested based on risk assessment and type of cell line (ex. optional for well-characterized cell lines?)	
EFPIA	1389	1390	Annex 7	Minor: It would be good to discuss this sentence in more detail. CHO cells may give a RT background signal , due to low levels of RT activity. Alternative, a co-cutlivation test is performed using sensitive cell lines with different read outs, e.g. cpe or PERT/PBRT.	
PPTA	1389	1389	Table A-5	Footnote e) "*In addition, a PCR-based RT assay (PBRT) assay, for example, …* "	Proposed change: *"In addition, a PCR-based RT assay (PBRT) assay, for example, …"*
EFPIA	1391	1394	Annex 7	Major Consensus Topic: Helper Virus Description/Defintion Major: The specificity for the testing requirements for residual helper viruses is appreciated, but is now proposed for each purified bulk, which is more restrictive than that proposed in Draft 1 and the requirements for cases C and D. Draft 1 had indicated a risk-based approach, with expectation for virus clearance. Can we clarify the basis for this expectation for residual helper virus testing on every batch versus a risk-based approach, where future technological advances for ATMP purification may be possible over lifetime of the guidance. Similar comment applies to Table 4.	Align requirements for purified bulk testing with Case C, D

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1391	1392	Annex 7	Define “campaign”, see comment for lines 430 - 432, Section 6	
EFPIA	1393	1394	Annex 7	Minor: Consider explicitly adding the option to use he QPCR testing for this test because this test is more appropriate for quantitative analysis.	
EFPIA	1394	1394	Annex 7	Major Consensus Topic: Helper Virus Description/Defintion Section 7.2, Table A.5: Propose to replace "absence" in footnote with "biologically irrelevant levels of helper viruses should be confirmed for each purified bulk (Case F, Table 4). "	See column F
Charles River Laboratories	1395	1403	Annex 7	footmote g and related row in table A-5: It is stressed that cells and supernatants of MCB/LIVCA and of unprocessed bulk harvest should be tested. Does this require seperate tests for cells and supernatant each or can one test be used on cell lysates?	
EFPIA	1395	1395	Annex 7	Major Consensus Topic: Replication competent virus testing It is the opinion of Janssen that the statement in line 1395 is not correct. Whether replication competent virus forms is fully dependant on the vector design and requires sequence homology between the E1 region of thevector and the E1 gene of the complementing cells. When there is no sequence homology, homologous recombination cannot occur and this effectively rules out the possibility that the vector can acquire or re-acquire replication competency.	Replication Competent Virus (RCV), depending on vector design, may develop at any step during manufacturing
EFPIA	1397	1398	Annex 7	Section 7.2: Table A-5: Sentence in footnote is not clear: The manufacturing stages and test methods are when applicable and product dependent.	Manufacturing stages and test metods are product dependent, when applicable.
EFPIA	1397	1397	Annex 7	Major Consensus Topic: Replication competent virus testing As indicated in row 16 above, testing for replication competent viruses should be made based on a risk assessment.	....for recombination or for the vector virus to revert to parental or wild type phenotype. RCV testing may not be required on each unprocessed bulk harvest or at each drug substance/final lot based on a risk assessment. The manufacturing stages and test .....
EFPIA	1397	1398	Annex 7	Major Consensus Topic: Replication competent virus testing Sentence in footnote g does not make sense.	EFPIA suggest to remove or change to "The manufacturing stages and test methods are product dependent, when applicable".

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
Charles River Laboratories	1404	1405	Annex 7	footnote h: A: Why not recommending other methods like NGS in such cases instead of analyzing control cell cultures. The safety value of testing control cells for some of these product types (e.g. AAV, VLP) is low considering further manipulation usptream of the manufacturing process. It should be a final solution only if other methods like NGS cannot be applied for interference reasons. B: footnote h is not relevant for in vivo unprocessed bulk testing (see table A-5)	Footnote h: A: "When in vitro/in vivo assay interference may occur other methods like broad NGS should be applied. Testing of control cells should be considederd only if alternative methods fail too." This recommendation would be more consistent with recommendation made at footnote a (If viral vectors and viral vector-derived products cannot be neutralised, a validated alternative 1376 assay can be used.) B: remove footnote h in the table for in vivo testing on unprocessed bulk
EFPIA	1404	1405	Annex 7	Major Consensus Topic: Control Cells Testing Guidance Major: Further guidance on control cells is needed. We appreciate the inclusion of a footnote stating that control cells are used when assay interference may occur. However more guidance is needed on when control cell testing is required, and how to perform the control cell testing. We believe it is not standardly required for AAVs (unless using helper virus like adeno or herpes simplex virus that would be detected by the in vitro assay) and suggest the guidance is clearer on that.	Please clarify when it is needed, when it is not. Proposal to include such narrative within the table cell itself. Proposal: When it is not feasible to perform standard virus testing due to assay interference (i.e. when the type of viral vector produced is detected by the in vitro or in vivo assay, or when using a helper virus like adenovirus or herpes simplex virus that can be detected by the in vitro or in vivo assay), either controls cells or NGS are used.
EFPIA	1404	1405	Annex 7	Major Consensus Topic: Control Cells Testing Guidance Annex 7, Table A-5; in vitro viral testing for adventitious agents is challenging when the feed stream already contains helper virus - 'control cells cultured in parallel are tested at the virus seed and unprocessed bulk harvest' does not sufficiently clarify how to meet this challenge. This problem may go away with NGS	
EFPIA	1404	1405	Annex 7	Major Consensus Topic: Control Cells Testing Guidance Major Table A-5: Information on how to test control cells is missing. Harmonization needed, since EP 2.6.16 has very specific control cell testing plan which is more or less a modified in vitro virus assay, whereas FDA 2020 guidance says to test any test that should have been tested on harvest if it was feasible (this would be a standard in vitro and in vivo, if applicable).	Please include guidance on how to test the control cells. Also would be helpful to include guidance on control cells in main text, and not only as footnote

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1404	1405	Annex 7	Annex 7, table A-5: Major: Superscript "h" states, "When assay interference may occur control cells cultured in parallel are tested at the virus seed and unprocessed bulk harvest stages". This suggests the only options, where-as superscript 'a" and "b" offer more flexability for alternative options. As "h" specifically points to UPB and viral seeds can we place "h" next to "a" and "b" in the table and not in the cell under the viral seed and UPB headers? It suggest a parallel culture preference where-as it should be complementary or substitutional. same for in vivo line below, move "h".	Move "h" superscript to "InVitro assays or NGS" cell and to "In vivo assays or NGS" cell next to "a and b" superscripts. Replace footnote h line 1404: "When assay interference may occur control cells cultured in parallel are tested at the virus seed and UPB stages". By "When assay interference may occur control cells cultured in parallel are tested at the virus seed and UPB stages, if options "a" and "b" are not applicable." [Suggest "Use either control cells or NGS"]
EFPIA	1404	1405	Annex 7	Major Consensus Topic: Control Cells Testing Guidance Consider to add that control cells will be tested for CPE, HAD and/or HA (as would be the same read out as on the traditional in vitro indicator cell lines)	
SGS Vitrology Ltd	1404	1405	7.2, Table A-5 footnote	For clarification, e.g. is it an expectation that the control cells are cultured for a minimum of 14 days (as required e.g. in Ph. Eur. 2.6.16), or collected at point of harvest?	Could a statement on expected culture duration for control cells be included?
Charles River Laboratories	1408	1408	Annex 7	footmote j: see comment for lines 1384-1385 (antibody production assays). Antibody production assays or alternative methods (NAT, targeted NGS) should be applied on rodent cells used for virus seed production and regarded mandatory based on other chapters (and table 1). Why is this option (no testing on cell substrate level) considered? Interference is more expected on the virus seed testing than on the related substrate.	Removing the footnote j same as suggested for footnote d and the related row in table A-5
EFPIA	1410	1413	Annex 7	Why "may originate" and not only "originate"?	
EFPIA	1417		Annex 7	Minor: Recommend to provide an example of what the alternative stages could be.	
EFPIA	1427	1427	Annex 7	Major Consensus Topic: Helper Virus Description/Defintion Clarification on viral clearance expectations for protein-expression virus vectors if similar viral clearance expectations exist for helper viruses. Recommend to explicitly include protein-expression virus vectors in the text.	Proposed revised text as follows "and if possible, the relevant helper virus and/or protein-expression virus vector"

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BioPhorum	1427	1429	Annex 7	if possible' does not reflect the current state of things. Industry is not clear on any situation when this would not be possible. Additionally, maintaining 'if possible' means that the message is different from line 1441 onwards, and would therefore create confusion.	
Pall Life Sciences	1431	1433	Annex 7	Alternatively has no relevance in this sentence as there is no step where this is an alternative for. Chromatography is a published and accepted technique for clearance of viral contaminants which are not of similar properties to the viral vector and should be given equal or greater standing compared to the virus filtration and low pH hold.	"Chromatography steps can provide virus clearance for multiple contaminant viruses with different surface properties to the viral vector. In addition, virus filtration may be suitable for small viral vectors such as AAV or nanoparticle-based vaccines when removal of larger viruses can be based on the size exclusion."
Lonza	1433	1434	Annex 7	As per the comment above on dose risk ! This paragraph refers to Section 6, which refers to Annex 5 for calculation of estimated particles per dose. However, it is not possible to perform such a calculation for AAV, since the TEM testing that forms the start of the calculation is not possible for AAV.	Propose that clarification is added to confirm that the estimation of particles per dose is not relevant for AAV, and that just providing reduction factors per step is sufficient to demonstrate viral clearance. Conversely, if the estimation is required, request that further information is provided on how this may be achieved (e.g. using TEM result from control culture or is it even necessary ?).
Charles River Laboratories	1433	1433	Annex 7	Missing some comments on usage of chromatography/ precipitation steps for viral clearance claim. One could refer to chapter 6.3 (lines 661-672 specifically) or outline the pro and cons of chromatography / precipitation steps. We find it usefull to comment on chromatography steps here as they are frequently applied in vector/VLP purification and can have significant viral clearance capacity for relevant/specific/helper viruses or general model viruses in certain cases. Even though the robustness of chromatography steps for general virus removal might be limited documentation of some removal capacity contributes to the safety of the product significantly. Prior knowledge principles can be applied too.	
EFPIA	1441	1441	Annex 7	Major Consensus Topic: Helper Virus Description/Defintion Clarification requested on the classification of protein-expression virus vector as process-related contaminant. If so, then recommend to revise text accordingly.	Proposed revised text as follows "Helper viruses and/or protein-expression virus vectors are considered process-related viral contaminants"
EFPIA	1441	1442	Annex 7	Major Consensus Topic: Helper Virus Description/Defintion Provide some guidance on minimum log clearance expected because “excess of helper virus clearance” is rather vague?	

Name of organisation or individual	Line from	Line to	Section number	Comment and rationale	Proposed changes / recommendation
EFPIA	1442	1442	Annex 7	Major Consensus Topic: Helper Virus Description/Defintion Clarification requested on expectations for viral clearance for protein-expression virus vectors.	Proposed revised text as follows "processes need to ensure an excess of helper virus and/or protein-expression virus vector clearance."
EFPIA	1445	1445	Annex 7	Section 7.3: After "closed systems", use "as applicable"... as downstream process steps, while not supporting further virus growth, may not always be closed, for example.	See column F