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Via Electronic Mail

30 September 2009

World Health Organization (WHO)
Avenue Appia 20
1211 Geneva 27
Geneva, Switzerland

Ref: *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks (Draft, 8 July 2009)*

Attn: Drs. Ivana Knezevic & John Petricciani, WHO Study Group Chairs

Dear Drs. Knezevic & Petricciani

PDA is pleased to provide comments on the revised WHO recommendations for evaluation of animal cell substrates. Our comments were prepared by an international expert committee with practical scientific experience in manufacturing and regulation of biotechnology derived products and the cell substrates used in for such in such processes.

In our regulatory consultations PDA attempts to restrict our commentary to scientific and technical issues. Many of our comments on this document are based on the principle of harmonization and consistency with existing guidance, e.g. ICH guidelines, and with accepted scientific terminology and knowledge. Where appropriate, we offer alternative recommendations to make the guidance more useful to all parties. Our comments are presented in the table format requested by WHO.

If you have any questions please contact me or James C. Lyda (lyda@pda.org) who did the staff work with our committee.

With very best regards,

Richard M. Johnson
PDA President

cc: R. Levy, G. Roessling, J. Lyda, R. Dana, K. King

Attachment

PDA_COMMENTS_WHO_CELL-SUBSTRATE_FINAL_2009_09_29

PDA Commentary on the WHO document,

"Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products (Draft 8 July 2009)"

PDA Review and Comment Committee:

Anthony Cundell, Schering-Plough
Jens-Peter Gregersen, Novartis
Leonard Hayflick, University of California San Francisco
Linda Hendrick, Centocor
Arifa Khan, FDA Center for Biologics Evaluation and Research
Kathryn King, FDA Center for Drug Evaluation and Research (Committee Chair)
Robert Kozak, Bayer
Zhong Liu, Schering-Plough
Barbara Potts, Consultant
Michael Ruffing, Boehringer-Ingelheim
Sally Seaver, Seaver Associates LLC
Glyn Stacey, National Institute for Biological Standards and Control
Dominick Vacante, Centocor
Hannelore Willkommen, Regulatory Affairs and Biological Safety Consulting
Martin Wisher, BioReliance
Ruth Wolff, Biologics Consulting Group

PDA Staff Support: Jim Lyda & Iris Rice

Sections	Page/ line No/ Text	Comment	Suggested amendment
General Comments	Whole document	The WHO guidelines should recommend the use of harmonized tripartite testing where these tests are available. The importance of such harmonization of testing is highlighted by the need for biologics manufacturers to be able to produce influenza vaccines rapidly for a pandemic H1N1 influenza outbreak.	Where possible harmonization with tripartite guidelines (ICH) should be attempted throughout the document.
	Whole document	The document should be consistent with regards to terminology used and guidance given. An example of inconsistent terminology would be the mixed use of ECB and EOP. An example of mixed guidance would be the time at which cells should be examined for tumorigenicity. This is not consistent throughout the text. We have highlighted specific inconsistencies in the comments that follow.	Maintain consistency in terminology and guidance throughout the document.
	Page 6 prior to the introduction	This document needs a glossary that includes a list of abbreviations and their meanings.	Include a glossary of abbreviations used throughout the document.

Introduction			
Historical Overview	Page 12/ Line 263 for HIV vaccines	Should distinguish from HIV vaccines in HeLa cells	Suggested text: ***for adenovirus-vectored HIV vaccines***
Scope	Page 14/ Line 307	It is unclear as to when these recommendations apply. Are they intended to be applicable only to approved products or to products in the clinical trials?	Recommend excluding clinical trial products.
	14/317 "… to reduce rcDNA in biotherapeutic products to < 10 pg per dose because they can be highly purified."	The units in this sentence (**pg**) are out of step with common practice and should be revised to <10**ng**/dose	Suggested text: to reduce rcDNA in biotherapeutic products to < 10 ***ng*** per dose
	14/319: "Live viral vaccines, on the other hand, are difficult to purify without a significant loss in potency, so that the amount of rcDNA in those final products may be significantly higher than 10pg/dose."	This statement applies not only to live viral vaccines, but may also be applicable to inactivated vaccines (where it may apply to a greater extent due to higher purification/less dilution). Additionally, the units in this sentence should be **ng** (see comment 14/317)	***Delete ~~"Live"~~:*** Viral vaccines, on the other hand, are difficult to purify without a significant loss in potency, so that the amount of rcDNA in those final products may be significantly higher than 10**ng**/dose.
	15/333. "these recommendations exclude"	A list of product classes (including types of vaccines) encompassed by the scope of the document would be valuable to the reader. Also,	Need more detail on what the recommendations include as part of the scope, specifically on the points suggested under comments.

		regarding the types of substrates that are within the scope, are embryonated hen's eggs used for vaccine production included? If so, this should be explicitly stated on page 23.	
	15/333 …microbial cells	Does not accurately indicate what is intended	Add text: ..microbial cells ***(bacteria and yeast).***
Definitions	17/370 definitions	No definition of "biological medicinal products" was provided.	Provide a definition for "biological medicinal products".
	17/370 definitions	No definition for "biotherapeutic" was provided.	Provide a definition for "biotherapeutic"
	17/372 Cell Bank "… aliquot of a single pool of cells".	Definition of cell banks needs further clarification.	Add text: ***"..which were frozen at the same time."***
	18/377 Cell Line	The definition needs modification. The small print really is the definition. However regarding the second sentence in small print, not all the cell types present in primary cells will necessarily be present in a cell line.	Suggested definition: ***"Cloning and subcloning steps may be used to generate a cell line. The term cell line implies that cultures from it consist of lineages of <u>some</u> of the cells present in the primary culture"***
	18 /381 Cell Seed " frozen at -160° C or below"	This might be too stringent, when the value is to be validated including technical and measuring/calibration deviations. We measured	Suggested change: ….***<u>frozen at -140°C or below preferably</u>*** in the vapor phase

		-160.4^{o}C in the upper gas phase in several tanks.	
	18/386 Diploid Cell Line	If they are derived in a suitable serum-free medium, DCL do not necessarily have to have finite lifespan. (Loo, D.T., Fuquay, J.I., Rawson, C.L. and Barnes, D. W., 1987, Extended culture of mouse embryo cells without senescence: inhibition by serum. Science 236, 200-202)	Suggested text: "***According to current understanding***, a cell line having a finite *in vitro* lifespan….." Add to small print. ***"It may be possible to isolate diploid cells that have an infinite in vitro lifespan if serum is not used. (Science (1987), 236; 200-202). This is an important concept especially for stem cells. With the right factors in the media or from cell extracts, scientists might learn to propagate them.***
	18/395 DNA Infectivity	Just because DNA integrates, does it "promote" proliferating clones?	Change text to: ***"may" promote***
	19/403 "In some cases production cells are expanded under pilot scale or commercial scale conditions".	Why "in some cases production cells"?	Delete "~~in some cases~~"
	19/415 Latent Virus	A definition of "latent virus" should be included	Suggested definition: ***"A virus that is present in a cell, without evidence of active replication, but with the potential to***

			reactivate, which is considered to be microbiologically latent"
	20/432 – 444 Parental Cells	We believe a more precise definition is necessary and that some of the examples given are confusing and/or incorrect. We suggest deleting the current definition and small print (lines 432-444) and replacing them with the suggestion provided.	***"A population of cells from which a derivative cell population has been obtained that differs in one or more characteristics. Manipulation to obtain the derivative population may be as simple as the expansion of a cell culture to produce a cell seed, or a more complex activity such as developing a hybridoma. For recombinant cell lines, parental cells are defined as the cells prior to the introduction of the DNA construct."***
	21/456 Population doubling	Grammatical point: "collection of cells that doubles..."	Suggest changing text to: ***"Collection of cells that has doubled in number."***
	21 /459 Log (N/N0) x 3.33	1/Log2 = 3.322	Log (N/N0) x 3.32
	21/466 "subcultured at least…"	replace "subcultured" with "passaged"	Suggested text: ***...passaged*** at least several times.
	21/472 TSEs	GSS should be defined	Define GSS
	22/487 Working Cell Bank	Further clarification is needed. See suggested text.	Suggested text: …containers appropriately

			cryopreserved ***on the same day from the same pool of cells and stored at -140° C or below.***
	22/491 Working Cell Bank	When would transfer not be acceptable?	….storage site ***is acceptable***
General considerations	23/493 Tissues are not included in PCCs	What about the use of whole eggs for flu vaccines?	If eggs are included in the scope, it should be explicitly stated in the document.
	23/506 "tissues disaggregated"	Grammatical point- see suggestion	Suggested text: "disaggregated tissues…"
	24/517 The number of viruses….	The meaning to the sentence beginning "The number of viruses…" is unclear. We recommend that it is deleted along with the preceding sentence and that the first sentence of the paragraph be merged with the next paragraph (see suggestion).	Suggested text: " PCCs obtained from wild animals usually show a high frequency of viral contamination. If PCCs are necessary for the production of a given biological…."
	24/524 for the absence of such viruses which can be detected	Grammatical point- see suggestion	Suggested text: ***"…viruses. Viruses can be detected by…"***
	24/525 "…antibodies to these agents."	Replace "agents" with "viruses"	Suggested text: "…antibodies to these ***viruses"***.

	24/527	A definition of "specific-pathogen free" should be provided in the definitions section.	Include a definition of "specific pathogen free" under definitions.
	24/527-529 The use of passaged cells…	In the context of primary cell cultures this sentence is unclear. Clarify what is being used and for what purpose.	Please clarify
	31/684 "as a cell substrate."	Add suggestion to the end of the sentence for clarity.	Suggested text: "as a cell substrate ***to produce vaccines or therapeutic agents***".
	32/697-703 The 1986 WHO SG reviewed…	This paragraph is unclear and does not add value to this section.	Delete paragraph 697-703. Begin the section with the paragraph starting on 705.
	33/719-720 ...measles and live influenza vaccines…	Was live influenza really produced in ALV+ eggs? Also measles?	These statements should be verified and supported with references.
	33/724-726 Rodents may harbor….	This sentence should be split into 2 sentences. Additionally, insect cells should be included in the paragraph, since they also express retroviral-like particles.	Suggested text: Rodents may harbour exogenous and endogenous retroviruses. Lymphocytic choriomeningitis virus and hantaviruses are exogenous viruses which have caused disease in humans.

	34/731-732 37/815 "Requirements" 39/851 "For products such as monoclonal antibodies…"	For clarity change "biotherapeutic" at the end of line 731 to "recombinant therapeutic protein products" Grammatical point- use lower case "r" -see suggestion This sentence implies that DNA spiking studies MUST be done. What is important is that residual host cell DNA levels in the final product are acceptable and accurately quantified.	Suggested change: ~~Biotherapeutic~~ ***recombinant therapeutic protein products*** "***r***equirements" Suggested text: For products such as monoclonal antibodies manufactured in tumorigenic cell substrates, it is necessary to ***demonstrate*** clearance ***(***removal and/or inactivation) of host cell DNA by the manufacturing process.
Part A. General recommendations applicable to all types of cell culture production	General Comments	The document appears to use a number of terms, such as 'cell(s)', 'cell culture(s)', and 'line(s)', interchangeably. This may cause confusion and lead to inaccuracies.	Terminology should be used consistently throughout the document.
A.1 Good manufacturing practices			
A.2 Principles of good cell culture practices	Section A.2 42/918. 2. absence of	Harmonization: Closer alignment with applicable Q5 documents Change contamination to	Section should include discussion on the benefits of a multi-tier banking system when the cell type permits (Paraphrase Q5D) Suggested text:

	microbiological contamination	agents to include endogenous viruses	Absence of microbiological ***agents to include endogenous viruses***
	42/919, "3. Stability and functional integrity on extended in vitro passage"	It is unclear what is meant by "functional integrity".	Functional integrity and the type of data required to demonstrate it should be defined in the document. Footnote 7 to table 4 might be an appropriate location.
	43 /928: "The four cell culture types …"	Define the "cell culture types" to which you are referring.	Suggested text: The four cell culture types, ***PCC, DCL, SCL and CCL***, …"
	43/932	Change "to" to "from"	"… substantially different ***from*** the …"
	43/936	Replace "changes" with "alterations"	"… cells may undergo subtle ***alterations*** in their biology in response."
	43/ 938	Add "and"	"Medium ***and*** specific additives …"
	43/940	Run-on sentence, split into two sentences.	"… of chemical composition and purity**.** Where relevant, biological activity should be determined before use."
	44/950 " Where complex biological reagents such as FBS remain necessary, they should be carefully controlled by pre-use selection of batches"	It may not be possible to develop relevant "pre-use" tests for all complex biological reagents; e.g. FBS used in initial stem cell isolation.	Suggest adding to line 951 following "batches": ***"This may not be possible in all cases (e.g. FBS used in initial stem cell isolation), however, it remains a point that***

			should be considered by the Sponsor "
	44/954 ….pH, temperature, and gas ….		Add humidity and does gas mean CO2?
	44/954-958 physical culture parameters	See suggested text	Suggested text for addition to line 956: …calibrated and monitored. ***This may not be applicable to very early development; e.g. novel cell lines first developed in academic laboratories.***
	44/963 manipulation of cell cultures	Emphasis should be on demonstrating a consistent process. Some systems will require extensive manipulations, e.g. microcarrier.	Suggested text to replace ~~"Care should be taken to minimize manipulations"~~: "An emphasis should be placed on demonstrating a consistent process".
	44/966-967 Cell harvesting	Does this mean trypsinization?	Please clarify
	45/972 A.2.2.2 Cryopreservation	Consider moving this section	Suggest inclusion of section A2.2.2 in Section A5 "Cryopreservation and cell banking".
	45/974	Other cooling rates may be suitable	
	45/980: " Cells are usually cryopreserved at temperatures of -	Same comment as page 18, line 381 (see comment above)	Suggested change: ….***frozen at -140^{o}C or below***

	160° C or below in the vapour phase of liqid nitrogen."		***preferably*** in the vapor phase
	45/981 "…preservation of cells should be carefully validated and controlled…"	It is not clear how preservation of cells would be validated; i.e. an MCB is a unique entity	Suggested text: "***The cooling cycle/equipment that is used in the preservation of cells should be validated. Procedures should be carefully controlled and exposure of the cells to cryoprotectant prior to freezing should be minimized. Viability should be confirmed prior to release of the bank***."
	45/982 "…the viability of each preserved batch checked immediately by recovery of a representative sample…"	In practice, we generally wait several hours or days to check viability to decrease risk of a temperature excursion occurring in the freezer.	Suggest deletion of the word ~~"immediately"~~ from this sentence.
	45/988 " Typically, such cells with be in the exponential phase of growth or subconfluent and with a high nucleus:cytoplasm ratio"	Determination of nucleus:cytoplasm ratio is subjective and not a measurement that many labs are equipped to make	Suggest deletion of "~~and with a high nucleus:cytoplasm ratio~~"
	46/1001 Therefore, cell manipulation should be minimized.	Add "and open processing steps"	Suggested text: "cell manipulation ***and open processing steps*** should be minimized"
	46/1005 use of antibiotics	antibiotics may be used in some cell line selection systems	Suggested text: …first passages of primary cell cultures. ***Additionally, antibiotics***

			may be used as in some cell line selection systems.
	46/1007 penicillin or other beta-lactam antibiotics	Addition to text.	Suggested text: "in production cell cultures ***of new products"***
	46/1010-1014	1010-1014 Run-on sentence, split into two sentences	Suggest: "Training in all cell culture processes is vital to ensure correct procedures are adhered to under cGMPs. Staff should be trained in the underlying principles of cell culture….."
	47 / 1018-1019: "Cell cultures should be prepared by staff who have not, on the same working day, handled animals or infectious microorganisms, … "	Is this realistic, particularly for small enterprises or for virus production and control laboratories?	Suggested text: ***"Whenever possible, maintenance of cell cultures should be separated from work with animals or infectious microorganisms, for example by separation of rooms or benches. Cell cultures should be prepared by staff, who have not on the same working day, handled laboratory animals or microorganisms unless appropriate cleaning and re-gowning measures are taken.***
	47/1020 Sick Staff	It is unlikely that sick staff member would know source of their infection and/or whether	uggested text for addition to line 1020: ***"Furthermore, cell cultures should not be prepared by staff, who are known to be suffering***

		the cell cultures would be permissive. Sick staff members should not perform cell culture operations	***from an obvious infection. The personnel concerned should periodically undergo a…."***
	47/1021	Not clear how this can be enforced and monitored	
	47/1030 rebanking	Adaptation to new growth medium/conditions such as serum- or protein-free media should not a priori require rebanking a new MCB. Historically this was the case, but it is not so anymore and is directly contradictory to Q5D (2.2.1). The emphasis should be on demonstration of ability to support production of consistent product, i.e. "If changes in the cell culture process do not affect product quality, it should not be necessary to rebank the MCB."	Suggested replacement text: ***"Whenever a cell culture has passed through a process that may significantly have an influence on its characteristics, it should be treated as a new cell line and should be renamed with a suffix or code to identify this. It should then be rebanked as a new MCB that is prepared from the post "treatment" culture. Treatments that may require such rebanking include cell cloning and genetic manipulation.***
	48/1042 pre-master cell banks	Initial banks of selected clones typically are prepared in R&D lab as soon as sufficient cell numbers are available to secure precious cell lines against	Suggest deletion of ~~"under cGMPs"~~ from line 1042. Amend to read "…for establishment of small pre-master cell banks under controlled conditions. A final selection will be

		potential loss. Thus, these initial banks are not prepared under cGMP.	made, often based on…."
	48/ 1051 Figure X "Simplified Schematic of Cell Line Development"	The figure is too generic (see comments below) and may cause confusion. Clone selection is not typically based solely on product quality; but on cell growth parameters and productivity. Evaluation of product quality from small scale culture may not be relevant to full scale production process. Selection criteria appear incomplete and inconsistent with previous text. Correct the label for Pre-Master Cell Bank.	Recommend: **Delete Figure.**
	49/1064 "cloning a cell culture…" 49 / 1066 – 1068: " …and evidence (including photographic records) that the new clonal cultures were derived from single cells. "	Please clarify "process of cloning a cell culture" Impractical proposal. How does one provide accurate and meaningful photographic records, (e.g. several hundred clones are made/screened) when it is not yet clear which clone(s) will be chosen later on? What is the evidence of a single cell on a photograph?	Suggestion deletion of reference to photographic records. New sentence: "The cloning procedure should be carefully documented; including the provenance of the original culture, the cloning protocol and reagents used."

	49/1068 cloning by limiting dilution	Sentence is not accurate.	Suggested text: "Cloning by ***one round of*** limiting dilution will not necessarily guarantee derivation from single cells; ***additional subcloning steps should be preformed.***
	49/1071-1077 characterization of cell clones	While some understanding of the clones is needed to choose the production substrate, the data required appear to be more in line with the ICH Q5B requirements for the chosen production substrate. Genomic evaluation of early candidate clones is not feasible. Genetic characterization including copy number, number of integration sites and message homogeneity is typically performing on cell cells from WCB expanded under pilot plant or full scale to the maximum in vitro cell age intended for commercial production (Q5B)	Please clarify.
	49/1077 …integrated DNA	Can also be episomal	Remove integrated
A.3 Selection of source materials of biological origins	50/1104 selection of source materials 51/1113-1119 approved source of animals-derived	"All Materials"	All material ***of biological origin…*** Recommend mentioning certificate of suitability for animal sourced materials.

	52/1136-1139 manufacturing	Facilities usually produce bovine and porcine products	Recommend including a note regarding control to prevent cross-contamination of one material with another (bovine in porcine product)
	53/1173	Serum is not routinely tested for mycobacteria. Viruses: would be useful if specific viruses were listed.	
	53/1174	Change “or” to “and"	“..and WCBs, ***and*** in the manufacture of biologicals.”
	53/1176	A list of potential zoonotic and/or oncogenic bovine viruses would be useful; particularly those that would not be detected by CPE or hemadsorption using bovine turbinate cells.	Provide a list of potential zoonotic and or oncogenic bovine viruses.
	53/1176-1178: …such as inactivation by heat or irradiation to ensure that any adventitious agents	It cannot be assumed that all adventitious agents that might be present in the serum can be inactivated by heat or gamma irradiation. Many viruses are very resistant to heat and also to irradiation. For example BPyV, circovirus and parvovirus are very stable. Inactivation of a broad range of viruses requires an extremely high dose of	Suggest: Delete ‘any’, ‘heat’, and ‘nonetheless’:

		gamma irradiation, which is not normally used.	
	54/1179 serum	This is a relative, not an absolute, target.	"… be inactivated ***to an acceptable degree.***"
	54/1182-1184 If the amount of contamination is greater than the inactivation (i.e., irradiation)	In principle this statement is correct, but it would be better to clarify that contaminated serum is only acceptable in rare cases. This might be better expressed in the proposed wording. Is it possible to provide a reference for testing (e.g. Ph.Eur.Monograph *Bovine Serum (2262)*	…the tests for adventitious agents should be performed prior to inactivation to enhance the opportunity for detecting the contamination. **If evidence of viral contamination is found in any of the tests, the serum is acceptable only if the virus is identified and shown to be present in an amount that has been shown in a validation study to be effectively inactivated. For serum that is not to be subjected to a virus inactivation /removal procedure, if evidence of vial contamination is found in any tests, the serum is not acceptable.**
	54/1193-1199 subsequent stages	Line 1195 is in conflict with line 1197	Improve clarity and meaning of use of serum during cell banking and production
	55/1209 such as NAT …	need definition	NAT (Nucleic Acid Testing)
	55/1220 Virus families…..		Add: circovirus and anelloviruses

	56/1244 and line 1267	add text to end of line 1244	"…***for that species and agents to be sought."***
	57 /1260 "Recombinant human trypsin derived from plants is available	Do not restrict recommendation to only one of many recombinant trypsins.	Suggested text" ***Recombinant trypsin is available ….***
		Check reference. If this is Sigma's Trypzean, it is bovine trypsin produced in corn	Check reference
	57/1264-1267 trypsin	Appears redundant with line 1247-1249	Consider deletion
	58/1274	Grammatical point- see suggested text	"…at the end of each culture period, ***but not less than 4-5 days after the last sub-culture"***
	58/1277	Addition to text	WCB or cells derived from WCB at maximum in vitro cell age
	58/1286..other porcine-derived materials	What does this refer to? Also, it implies that trypsin is only tested for PPV. Is this the intent?	Please clarify
A.4 Certification of cells	58/1289 testing of porcine derived materials		Add Hepatitis E virus
	59/1331, 1348 Certification of cells	"Certification" implies acquisition of a formal certificate, which could be problematic for Sponsors.	Suggest changing this to "Cell Line History", which would include information on the origin of the culture and how the cell line was

			developed.
	61/1344….culture conditions…		Add reagents (bovine, horse, donkey, sera)
	61/1349: origin of primary cultures		Add vaccinations
	61/1352-1354	Extensive testing is independent of cryopreservation.	Please clarify
	62/1363-65	Although flasks/containers may be observed for potential presence of microbial contamination prior to pooling to prepare cell bank, it is not feasible to actually perform bioburden test.	Suggested modification to sentence: ***"Prior to any culture pooling, cells should be observed for acceptability for production. Acceptability criteria (ie cell viability and number) should be established and should include observation for microbiological contamination.*** Please also include a discussion of pooling at risk for bioburden contamination if forward processing cannot be delayed.
	62 / 1367- 1369: " .. and ideally microbiological testing should be completed and passed before the cells are used. If appropriate, preparation of a cryopreserved	Almost invaribly testing takes much longer than the expected life span of primary cells. Primary cell banks may be an extremely rare exception (I	Recommend: delete quoted text.

	`primary cell bank` will enable completion of such quality checks prior to use of the cells for production	have not heared about one at all), and if these exist these may probably be better defined as diploid cell cultures.	
	63/1391	Flow cytometry can be used in lieu of immunocytochemistry	Add: ***flow cytometry or immunocytochemistry***
A.5 Cryopreservation and Cell banking	63/1403 cooling profile		"should be qualified; ***e.g. by prior use with same cryopreservative***
	63/1404 cooling profile		(e.g., documented in a SOP or batch record)
	64/1413 cell banks	Please refer to ICH Q5 documents for cell bank definitions	
	64/1423-1427 cell banks	No mention of stability program	Include the concept of a stability program for cell banks
	64/1424 viability of frozen cells	Generally wait several hours to check viability to decrease risk of temperature excursion in the freezer-redundant with 982 text.	Suggest deletion of the word "immediately" line 1424. New sentence "When a stock of cells has been frozen, a sample should be recovered to confirm it has retained viability…"
	64 and 79/1426, also 1713-21 evaluation of cryopreserved cells cells	Thaw of 3% of vials and evaluation at small scale will not result in confidence in production process based on use of a given cell bank. Rather, the stability and	Suggest: ***"Recovery of a sufficient percentage of vials representative of the beginning, middle and end of the cryopreservation process should be demonstrated to give confidence in the production***

		integrity of cryopreserved vials is demonstrated when vials are thawed for production and demonstrated to produce intended product at scale.	***process based on the use of that cell bank. Ultimately, stability and integrity of cryopreserved vials is demonstrated when the vials are thawed from production and demonstrated to produce the intended product at scale."***
	65/1437 generation of MCB	It may be necessary to use more than one vial of a pre-master bank to create a MCB	Suggested text "***One or more vials of the… are"***
	65/1453	It is generally not useful to evaluate final product for the presence of viruses	Add ***"Final products should be evaluated to demonstrate absence of bacterial contaminants; but it is generally not useful to evaluate final product for the presence of viruses or mycoplasma contamination. Unprocessed bulk is a more suitable test article for mycoplasma and virus testing, since these organisms are more likely to be present in unprocessed material."***
	66/1460 ampoules	"Cryovial" is more commonly used than "ampoule"	"cryovials or ampoules…"
	66/1461 geographically distinct storage locations for cell banks	Delete "widely" from line 1462. It is vague. See	Suggested text: "Storage of the MCB and WCB in at least two

		additional suggestions for text.	***geographically distinct locations to assure continued ability to manufacture product in the event of a catastrophe***. "
	66/1467 transfer of cryopreserved cells	Procedures for the transfer of cell bank ampoules between storage locations should be developed, and qualified use qualified shipping containers and probes to detect temperature excursions.	Suggest: ***"When cryopreserved cells are transferred to a remote site, it is important to use a qualified shipping containers and to monitor transfers with probes to detect temperature excursions."***
	68/1501	Spelling "isoenzyme"	
Part B Recommendations for the characterization of cell banks			
B.1 General considerations	70 / Table Murine myeloma (CHO, NS0, and SP2)	correction	***CHO and murine myeloma (NS0 and Sp2/0)***
	71/1547 …and some require the use of..	delete "require the use of" replace with "some use"	~~And some require the use of~~ some use
	71/Table Influenza vaccines	redundancy	repeated twice, consolidate
	71/Table Per.C6	correction	correct to: PER.C6
	72/1577 …safety of the product	addition to text	Suggested text: …the safety of the product ***and the cell substrate.***

	72/1583 ….tumorigenicity test, **then**… 72/1594 …certain CCLs express viruses such as retroviruses, herpesviruses and papillomaviruses	Change "then" to "and" Endogenous retrovirus is not a contaminant. Herpesvirus and papillomavirus are incorrectly stated: this may mean **genomes** not viruses for these.	Suggested text: "…positive in a tumorigenicity test ***and*** if the CCL…" Suggested text line 1594: ...certain CCLs may express ***endogenous retroviruses or may contain genomic sequences of adenoviruses, papillomaviruses or herpesviruses.***
B.2 Identity	75 Identity	Expanded characterization for recombinant therapeutic protein products should be discussed.	Suggested text: "***For recombinant therapeutic protein products, cell line identity testing should also include tests for vector integrity, plasmid copy number, insertions, deletions, number of integration sites, percentage of host cells retaining the expression system, verification of protein coding sequence and protein production levels."***
B.3 Stability			
B.4 Sterility			
B.5 Viability			

B.6 Growth characteristics			
B.7 Homogeneity	79/1713-1721, same comment as 1426 evaluation of cryopreserved cells cells	Thaw of 3% of vials and evaluation at small scale will not result in confidence in production process based on use of a given cell bank. Rather, the stability and integrity of cryopreserved vials is demonstrated when vials are thawed for production and demonstrated to produce intended product at scale.	Suggested text: "Recovery of a sufficient percentage of vials representative of the beginning, middle and end of the cryopreservation process should be demonstrated to give confidence in the production process based on the use of that cell bank. Ultimately, stability and integrity of cryopreserved vials is demonstrated when the vials are thawed from production and demonstrated to produce the intended product at scale."
B.8 Tumorigenicity	81/ 1763-1769 in vitro testing 82/1780 tumorigenic 82 /1791 … propagated to or beyond to the PDL limit for production should be examined for tumorigenicity	The paragraph on in vitro testing interrupts the discussion on CCLs. This paragraph should be moved to preserve the flow of the section. omission inconsistent with 86/1851 ("…propagated to at least three population doublings beyond the PDL limit for production should be examined for tumorigenicity."	Move paragraph on in vitro testing (lines 1763-1769) to follow the in vivo testing (ie insert the in vitro paragraph at page 86, line 1848) Include HEK293 as known to be weakly tumorigenic The position as to when the cells should be examined for tumorigenicity should be consistent throughout the text.

	83/1803 …nude mice***, and*** the SCID mouse, or …	Are both nude and SCID mice being recommended or either?	If the later is the case, remove the comma and change "and" to "or".
	88/1900 …inoculated intramuscularly or subcutaneously…	The rationale behind the route of inoculation (IM vs SC) should be clarified.	Clarify Rationale
	88/1901 …a maximum of 10^7 cells…	A specific ***minimum*** or 10^7 cells should be used	The ***minimum*** number of cells should be specified
	88 /1902 – 1908: If the cell line is found to be tumorigenic, the NRA/NCL might request additional studies to be done to determine the level of tumorigenicity. This can be done….."	It would be desirable to discuss the consequences of different levels of tumorigenicity. The risk assessments, measures, and consequences tend to be the same, irrespective of the level of tumorigenicity.	Please clarify why the level of tumorigenicy should be determined and discuss what the consequences of different levels of tumorigenicity might be.
B.9 Oncogenicity	92/1983 …(i.e. other than CHO and Vero	High passage Vero may still need to be tested. NS0 and Sp2/0 should be added to the examples of cell lines that should be excluded from oncogenicity testing.	Suggested Text: ***(i.e. other than CHO, low passage Vero, NS0 and Sp2/0…***
	92 /1995: " …similar amount of the same oncogene is 10^5 µg to 10^6 µg (i.e. 100 mg to 1 g).	100 mg to 1 g of what?	Suggested Text: Please specify: (i.e. 100 mg to 1 g of ***cellular DNA***).
	93/2005-2015 NIH3T3 assay	In our experience this assay not very reliable unless a dominant oncogene is present	Consider deletion of this paragraph, as it does not necessarily aid the reader, or stronger text on assay

			limitations
	94/2029 Newborn nude mice…	Age of newborn animals should be defined.	Specify age of animals: <3 d old
	95/2072 Cell lysate	Information should be provided regarding preparation of cell lysates to ensure reliability in virus detection.	Provide a description of how cell lysates should be prepared to avoid virus disruption and maximum virus release.
B.10 Cytogenetics	99/2172 …serial cultivation from the MCB through to 10 PDLs beyond that at the end of production.	Inconsistent with previous sections (82/1791 and 86/1851)	A consistent position with regard to when testing should be undertaken should be maintained throughout the document.

B.11 Microbial agents	102/2236 cell bank testing	Clarification is required. Testing of cells propagated to or beyond the maximum in vitro age intended for production needs to be done ***once for each commercial production process*** and not repeated for each WCB.	Add at line 2236: ***"Additionally, end of production cells propagated to or beyond the maximum in vitro age, should be tested once for each commercial production process. This does not need to be repeated for each WCB."***
	102/2245 …electron microcsopy, induction of a lytic infection by exposing the cells to special conditions…	Should include (chemical) induction for detection of latent or endogenous viruses. This may reveal a lytic or non-	Change text from "induction of a lytic infection by exposing the cells to special conditions" to ***chemical induction which may***

		lytic infection	***reveal a lytic or non lytic infections by latent or endogenous viruses***
	103/Section B.11.2/ 2266 Virus testing	Include concept of well-characterized cell lines (e.g. CHO, NS/0, Sp2/0) where reduced/no testing for retroviruses is required due to extensive characterization of endogenous particles and extensive safety record for products produced using these cell lines. Discussion in 2752-2760 is not sufficient	Suggest insertion of following text at line 2266: ***"Cell lines such as CHO, BHK, NS/0, and Sp2/0 have frequently been used as substrates for drug production with no reported safety problems related to virus contamination of the products and may be classified as "well-characterized" because the endogenous retrovirus particles have been studied extensively. Furthermore, the total number of retrovirus-like particles present in the harvest is evaluated quantitatively (TEM or QPCR) on a representative number of lots and retrovirus clearance is demonstrated with significant safety factors. Thus, in these situations testing for infectious retrovirus may be reduced; e.g. test one lot then discontinue testing, but repeat when there is a significant change in the cell culture process such as a change in scale. In all cases, samples derived from unprocessed bulk***

			are the appropriate test article and it is not usually necessary to test purified bulk as titers of any virus potentially present would be highest in unprocessed samples. Sponsors are encouraged to consult with NRA."
	Section B.11.2	PDL is used throughout section. However, for CCLs, it is also appropriate to quantitate culture duration by number of subcultivations at defined dilution ratio or time in days, as is stated in ICH Q5D.	Add the following to the end of the definition of PDL on page 21 of the definitions section: ***"For continuous cell it is also appropriate to quantitate culture duration by the number of subcultivations at a defined seeding density at each passage, or time in days."***
	106/2332 …although established rabbit cell lines are now acceptable (ref).		Provide reference for rabbit cell lines
	106/2322 ..established rabbit cell lines are now acceptable	Clarify what they are acceptable for.	Add: acceptable ***for virus detection***
	106/2332 Marburg virus and worker deaths	It should be made clear that monkeys were contaminated with Marburg virus and worker deaths were due to handling monkeys and not cells	Clarify this point in the text.

	107 B11.2.1, B11.2.1.1, B11.2.1.2, B11.2.1.3, B11.2.1.5 "Tests in animals and eggs"	We note that throughout this section the original purpose of the tests are identified. It might be useful to the reader to give examples of other types of viruses currently detected by these tests. The table should be qualified to indicate that these are examples, and that not every virus is a family would be detected by such tests.	Suggest that each section include a fuller list of the viruses detected by each animal as given in *"Draft Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases"* FDA CDER (2006)
	109/2403 suckling mice	Addition to small print	Add to small print: **in some cases 20 suckling mice are inoculated**
	113/2491 \	Should add FDA recommendation under in some countries	**Cite:** *"Draft Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the Prevention and Treatment of Infectious Diseases"* FDA CDER (2006)
	114/2516-2517 applicability	It seems that the applicability of testing in embryonated chicken eggs is limited to cells of avian origin. This does not comply with other regulations (ICH Q5A, EP 5.2.3., CBER 2006) where testing in embryonated	Propose deletion of 'avian' Edited text to read: ***Cell Banks: MCB or WCB; Cell Types: PCC, DCL, SCL, CCL***

		chicken eggs belongs to the in-vivo standard test system for detection of adventitious viruses.	
	Section B.11.2.2.2 Indicator Cells	Combining description of co-cultivated and inoculated cell cultures is not useful. Clarification is required concerning applicability of co-cultivation vs. inoculation approach	Please Clarify
	115-116/Section B.11.2.2.2 Indicator Cells	The use of 3 indicator cell lines is standard practice in the US EU and ICH guidelines. We recommend that the text be revised to reflect this. (ie same species and tissue type, human DCL and simian cells)	Change text to recommend the use of 3 indicator cell lines. Leave in the small print that in some countries 2 indicator cell lines (same species and tissue type and human DCL) might be acceptable.
	116 / 2577 - 2581 "In some cases it may be difficult to keep the cultures healthy for 2 weeks without subculturing. In those cases it may be necessary to feed the cultures with fresh medium or to subculture after two weeks onto fresh cultures in order to detect viral antigens."	Unclear what is really meant. Rapidly growing cells may require subpassaging earlier than after 2 weeks to maintain healthy cultures. Does "Subculture onto fresh cultures" mean that cells and culture fluid are inoculated onto new cells?	It may be necessary to feed the cultures with fresh medium. In some cases it may be difficult to keep the cultures healthy for 2 weeks without subculturing. In those cases it may be necessary to passage the cells, or to subculture cells and culture fluids onto fresh cultures in order to detect viral antigens.
	117/ 2593-2594 Insect viruses	Not sure if it is known that the reactivation of viruses occurs	Replace “generally” in line 2594 with “might be”

		under stress in "many instances"?	
	117-118/section B.11.2.2.3 insect viruses	Somewhere in this section should refer to the non-viral/bacterial/fungal infections of insect cells such as found in Drosophila ("X" agents)	Need input from Drosophila cell line expert
	117/2596	Use ECB or EOP throughout the document. Mixed use is confusing. For example ECB is used shortly after on page 119 line 2633	***Either*** EOP ***or*** ECB should be used consistently throughout the document, not both.
	118-119/ 2622, 2633 TEM	TEM of MCB only should be sufficient. To be consistent with Q5A, instruction should be provided that if WCB is examined in lieu of MCB, then each WCB will need to be evaluated.	Suggest: At least 200 cells from ***either*** the MCB ***or*** the WCB ***as well as EOP*** cells are examined…
	118-119/2621-2630 TEM	LOD cannot be determined for detection of retrovirus particles in thin sections of cells. Rather, LOD for detection of RVLP is determined in pellet produced by ultracentrifugation of culture fluid from production lot(s) (not from direct evaluation of MCB or WCB) and this value is used determine maximum titer of retrovirus potentially present	This section should be revised. See comment to the left.

		when no RVLP are actually observed (see B.11.2.3.2). This section is mixing up the two separate types of evaluations, both which use TEM.	
	118-119 TEM	Suggest additional point for inclusion in this section (perhaps page 119/line 2630)	Suggested text: ***"For cell lines known to endogenously produce RVLP, it is important to determine the viral load in the unprocessed bulk harvest in order to ensure that a sufficient degree of clearance is achieved by the downstream virus purification methods."***
	119/2638 while TEM is fairly insensitive…	Cite Reference and add suggested text. Khan A.S. et al., Proposed algorithm to investigate latent and occult viruses in vaccine cell substrates by chemical induction, Biologicals 2009; 37: 196-201	Cite reference in comment column and add text in parentheses to line 2640: …microbial agents of many types ***"However, chemical induction may be used to enhance detection of endogenous and latent viruses by TEM" (Khan et al reference).*** TEM also can be used…
	120/2671 …PERT assay for reverse transcriptase or TEM to reveal their presence.	Cite reference and add suggested text: Khan A.S. et al., Proposed algorithm to investigate latent and occult	Cite reference in comment column and add to the end of line 2671: "***Chemical induction may be used for enhance***

		viruses in vaccine cell substrates by chemical induction, Biologicals 2009; 37: 196-201	***endogenous retrovirus detection prior to PERT, TEM or infectivity assays (Khan et al).”***
	120/2661 …EAV, Chinease Hamster Ovary cell line gamma retro-virus)	“gamma-retrovirus”	Please provide a reference for “gamma retrovirus”.
	121/2676 …the substrate and cannot be demonstrated…	Change AND to THAT	Suggested text: …the substrate ***“that”*** cannot be demonstrated to be cleared…
	121/2677-2680 …made in cell substrates that produce retroviral particles (ie CHO cells) is validated to provide adequate viral clearance.	It should be acknowledged that the use of NS0 und SP2/0 cells is well established and no safety concerns are associated with this cell substrate even if testing for infectious retroviruses reveals a positive result. This should be mentioned in this paragraph in order to support the first sentence of this paragraph.	Add suggested text: …made in cell substrates that produce retroviral particles (i.e. CHO cells) **or infectious endogenous retrovirus (i.e. NS0, SP2/0 cells)** is validated to provide adequate viral clearance.
	121/2682-2684 Chick embryo fibroblasts…	Production of defective retrovirus particles with reverse transcriptase activity is not restricted to CEF, e.g. reverse transcriptase activity can also be detected in CHO cells. ***Suggest delete “are a special case in that***” as it implies it is the ONLY case and ***clarify why***	Suggested text line 2682: **“Chick embryo fibroblasts contain defective retroviral elements…..”** AND suggest addition to line 2684: “…subject of many studies and WHO consultations ***because they are used for live viral vaccine production.”***

		they have been the subject of many WHO consultations.	
	121/2691-92 …non-infections endogenous retroviral particles is masking an infection retrovirus contamination	An infectivity test can reveal the presence of infectious retrovirus; but it would not reveal "masking" of infectious retrovirus by non-infectious retrovirus-like particles.	Please clarify this statement. Are you suggesting that a manufacturer does spiking studies to look for masking by non-infections RVLPs? We are not aware of anyone doing this on a routine basis.
	121-122/B.11.2.4.2 Reverse Transcriptase Assay	See suggested text	Add to line 2719: ***"The RT assay is not necessarily required when retrovirus has been detected by other means, e.g TEM, infectivity assay (ICH Q5A, B1 and Table 1). However for some situations, such as CEF which express retroviral elements and are used to produce vaccines, it is necessary to show that there is no increase in RT activity between the initiation of the culture and the EOP."***
	122/2710-14 RT assay	Further clarification regarding interpreting results of RT assay with caution would be useful.	Add to line 2714: ….in this regard. ***"Since RT activity can be associated with the presence of defective retrovirus-like particles and since polymerases other than reverse transcriptase can result in apparent RT activity, a positive***

			result in an RT assay is not conclusion evidence of the presence of infective retrovirus." Positive results may require….
	123/2728-2731 addition to current text	The usefulness of the PCR or other specific tests is explained if the PERT test gives unclear results. It may be beneficial to mention here also that quantitative PCR may also be used for the determination of RVLPs in bulk harvest as an alternative test for TEM.	Suggest addition to the end of line 2731: ***"Molecular methods, such as PCR, may also be used for quantification of retrovirus like particles in the production harvests provided that the method is validated accordingly. Consultation with the NRA/NCL regarding the acceptability of this approach is recommended."***
	124/2747-49 "For non-murine retroviruses, test cell lines should be selected….	Examples of test cell lines appropriate for the detection of murine, as well as, human and non-human primate retroviruses would be useful. Additionally the reference at the left, which provides examples of target cells that can be used for retrovirus detection, should be cited.	We suggest the addition of 5-6 example cell lines that can be used for detecting retroviruses and the citation of the following reference: Khan AS, et al., The reverse transcriptase activity in cell-free medium of chicken embryo fibroblast cultures is not associated with a replication competent retrovirus, J Clin Virol 1998; 11:7-18.
	124/2755	Typo: Change SP0 to Sp2/0	"Sp2/0"
	124/2755: Murine and other rodent cell lines (CHO, NS0,	Many studies confirmed that CHO cells express retrovirus	Suggested text line 2755: ***"Murine and other rodent cell***

	SP2/0) or hybrid cell lines containing a rodent component should be assumed to be inherently capable of producing infectious retroviruses.	like particles, but not infectious retroviruses.	***lines (NS0, SP2/0, CHO) or hybrid cell lines containing a rodent component should be assumed to be inherently capable of producing infectious retroviruses or non-infectious retrovirus like particles."***
	124/2757-2760 Therefore, the extent of testing for specific retrovirus may be reduced provided that the purification process has been validated for sufficient viral clearance. In such cases, the clearance (removal and/or inactivation) of such retroviruses during the manufacturing process should be quantified.	The sentence beginning "Therefore, the extent of testing…" is misleading, because testing needs to be performed to determine the level of contamination, the stability in the expression of retroviral particles/infectious retroviruses and the absence of adventitious retroviruses if relevant. We propose deleting this sentence and altering the text of the following sentence.	Suggested changes line 2757-2760: ~~Therefore, the extent of testing for specific retrovirus may be reduced provided that the purification process has been validated for sufficient viral clearance~~ In such cases, the clearance (removal and/or inactivation) of such retroviruses during the manufacturing process should be quantified **and provide sufficient clearance**.
	125/2775: Generally, once the MCB or WCB has been demonstrated to be free of selected viruses, it might not be necessary to test the cells at later stages (i.e. at the production level) if such viruses could not be introduced readily during culture.	This statement is misleading and may lead the reader to believe that testing on the unprocessed bulk harvest is not necessary, when in fact it is required. Additionally, the EOP cells need to be tested once. See suggested text.	Suggested text line 2775: "Generally, once the MCB, **WCB or EOP cells have** been demonstrated to be free of selected viruses, it might not be necessary to test the cells at later stages (i.e. at the production level) if such specific viruses could not be introduced readily during culture. **Production harvests however are subject to routine**

			testing as described in B11.2.2.2."
	125/2784 tissue source and medical history of the donor	This section should indicate value of medical history ***if available***	Expand section to indicate value of medical history.
	128/ line 2842 Biological starting materials should be characterized…	See suggested text	Suggested text to add to line 2842: "Biological starting materials should be characterized ***...and if possible physically treated. Many starting materials can withstand heat at 121^{o}C for 20 minutes that will inactivate all extraneous infectious organisms or high temperature short time (HTST) that will inactivate all Mollicutes (Mycoplasmas, Acheleoplasmas, and Spiroplasmas) and many viruses. Gamma irradiation at 30kGy can inactivate many viruses that contaminate calf or fetal bovine serum. This dosage has also been reported to inactivate Mollicutes in serum but a higher dose (30-60kGy) has been reported for the inactivation of Mollicutes in tryptic soy broth powder."***

	128/ 2842-2850 Biological starting materials	Biological starting materials are not defined in the definition section. The compendial mycoplamsa tests recommend using positive control mycoplasma cultures appropriate for the source of the material tested including Spiroplasma control strains when testing insect cell lines. This point is more important than singling out Spiroplasma. We are unaware that the screening for Spiroplasma per se is ever extended to plant-derived materials.	Provide a definition of "biological starting materials" under definitions. See comment, suggest modifying the text. Additionally, because the comment regarding Spiroplasma testing on plant derived materials is different that what is currently generally practiced, the WHO should give a reference or provide justification for this statement.
	128/Line 2850 …account for the need for repeat testing to deal with potentially false-positive results.	See suggested text	Suggest adding to the end of line 2850: ***"…..and a pre-qualification plan for reagents used in the tests."***
	128/2852-54 mycobacterial testing	*Why is M Tuberculosis* singled out? *Mycobacterium avium* contamination has been reported as an interacellular bacterial contamination of mammalian cell lines and may be a greater risk than *M. tuberculosis* There are numerous reports that *M Scrofulaceum* is a common contaminant in plant cell culture	Suggest widening beyond *M. Tuberculosis*

		and could be an issue for reagents of plant origin. The slow growth in standard bacterial culture systems could lead to such contaminants being missed.	
		Lysis of the host cell may be necessary for Mycobacterium recovery.	Add text to the end of 2854: ***"Lysis of the host cell may be necessary for Mycobacterium recovery."***
	129/2856-2864 bacterial and fungal sterility	The WHO methods in Requirements for Biological Substances No 6 are dated. The ICH Harmonized Tripartite Guideline *Derivation and Characterization of Cell Substrates used for Production of Biotechnological/Biological Products* Q5D Step 4 version dated 16 July 1997 recommends the testing of 1% of the total number of MCB vials but not less than 2 vials using the current methodology described in the compendial sterility test, i.e. USP <71> *Sterility Tests*.	Recommend harmonizing the document, potentially through citing ICH guidelines where appropriate.
	129/2859-64 "For the MCB and the WCB, the test is carried out…"	The sterility test should be performed on material taken directly from the vial without the growth of cells. It should not be necessary to evaluate cell	Delete from lines 2859 and 2862: ~~the test is carried out using for each medium 10mL of supernatant fluid from cell cultures. In addition~~

		culture fluids from MCB and WCB when the filled containers are evaluated directly, provided the absence of bacteriostasis/fungistasis has been demonstrated for cryopreservative.	
	129 and 130/mollicutes, mycoplasma and acholesplasma, spiroplasma and other mollicutes	Pull these sections together. Spiroplasma is a member of Mollicutes,as are Mycopalsmas and Acholeplasmas. The trivial name for all Mollicutes is mycoplasma.	Revise text to reflect comment.
	129/2871-2876 Mollicutes	See suggested addition to text	Add text to line 2873 immediately after …contaminant of cell cultures. ***"For example, industry experts estimate that the mycoplasma contamination rates ranges from 15 to 30% in secondary cell cultures and 1% in primary cell cultures. After controlling Achoeplasma laidlawi, from animal-derived serum by 0.1 micron filtration, the industry moved from animal-originated media to plant peptone-derived cell culture media to avoid spongiform contamination resulting in an unanticipated upsurge in plant-derived mycoplasma contamination. Five species***

			Mycoplasma orale, M. fermentans, M. arginini, M. hyorhinus as well as Acholeplasma laidlawii account for approximately 95% of the identified cell culture contaminants so any screening method must detect a wide range of organisms. In addition to the potential pathogenicity, mycoplasmas compete for nutrients, induce chromosomal abnormalities, interrupt metabolism and inhibit cell fusion of host cells."
	130/2878-2897 Spiroplasma	Spiroplasma are insufficiently unique to warrant a separate section.	Add section on Spiroplasma to a general section on mollicutes.
	131/2909-2933 mycobacteria	Mycoplasma tests (Pharm Forum 35(1) Jan-Feb 2009) Biosafety level 3 practices, containment equipment and facilities are required for the cultivation of *Mycobacterium tuberculosis*. There are differences in media, sample size, incubation conditions and incubation time in the WHO	Recommend inclusion of a statement "in some countries" and a summary of the text in the comments column.

		document versus those recommended by the U.S. Center for Disease Control and Prevention and the Center for Biologics Evaluation and Research, FDA. For example, Middlebrook 7H10 agar is superior in recovery to Lowenstein-Jensen agar, which requires preparation from egg yolks, incubation under 10% CO_2 is recommended and incubation from 6 to 8 weeks. It is assumed that positive control recommended is *Mycobacterium bovis* Bacille Calmette-Guérin (**BCG**)	
	132/2940 TSE	vCJD is not slow versus classical CJD, suggest delete	Suggest alternate text line 2940: "***Transmissible spongiform encephalopathies (TSEs) are a group of fatal neurological diseases…"***
	132/2943 terminology in TSE section	PrPtse PrP	These should be added to a glossary alongside an explanation of the evolution of the terminology, as there are differences in the terminology used between this document and both the EMEA and FDA documents.
	133/2947 "Normal PrP…	Suggest addition to the text.	Suggest adding to line 2947: "Normal ***prion*** protein

	134/2971 134/2975 TSEs 134/2976 geographic location/TSE risk 134/2985 infectivity categories of tissues	PMCA should be defined Add statement regarding substitution of non-animal derived materials The OIE member country classification list (i.e., "Bovine Spongiform Encephalopathy Status of Members") for clarification for geographic location/TSE risk should be mentioned on line 2976. Following reference should be cited: WHO guideline on Tissue Infectivity Distribution in TSEs, 2006. This would clarify the tissue infectivity categories, A, B, C that are used. The EMEA uses the WHO tissue infectivity classification.	Protein misfolding cyclic amplification Add to the end of line 2975…***"and on substituting non-animal derived materials."*** Add mention of the OIE member country list to line 2976. Cite WHO guideline on Tissue Infectivity Distribution in TSEs.

B.12 Summary of tests for the evaluation and characterization of	138, graph in line 3060 "MCB PDL-12 2000 vials" AND	Is the figure of > 1000 vials realistic? For a WCB it may be adequate,	Omit number of vials for MCB. Alternatively use term " several hundred to >1000"

animal cell substrates	139 lines 3079 – 3080 : MCBs typically contain over 1000 vials"	but for a MCB?	
	139/3083 illustration	Use PDL instead of passage	Change “p” to PDL
	141/Tables 3 and 4	Applicability not described	Add applicability; PCC, DCL, CCL, SCL to table titles
	141/table 3 (AC)	Table 3 The distinction between the sterility and microbial agents tests is not clear. We recommend that testing be subdivided into Sterility (bacterial and fungal), absence of Mycoplamsa, absence of viruses, absence of mycobacteria.	Suggest: Divide table as follows Sterility (bacterial/fungal) Mycoplasma Viruses Mycobacteria
	141/Line 3099 and 3106 footnote 1 to tables 3 and 4	Footnote 1 is not clear. Insect cell lines may also have other Mollicutes as a contaminant. The way the footnote is worded implies that only tests for Spiroplasma must be done. Additionally, Spiroplasmas could contaminate plant starting material.	Testing for Mollicutes that include specific tests for Spiroplasma where insect cells or plant starting materials are used.
	Table 4: source of cells, initial cell evaluation and cell seed	The first 3 columns of table 4 (source cells, initial cell	List testing on Source of Cells, Initial Cell Evaluation, and on

		evaluation and cell seed) are not necessarily helpful to the reader. See comments below. With regard to Source of cells: Sterility testing may not be possible due to small amount of material available at this stage. Bioburden testing may be of limited use for source of new PCL and SCL; especially when antibiotics are used during tissue processing. These data will not be available for all "legacy" cell lines. Additionally extensive microbial agents testing not feasible due to small amount of material available at the time of procurement. It is doubtful if testing of the Cell Seed and Initial Cell Evaluation are realistic. Considering that testing for microbial agents may take one year and causes considerable cost, one may prefer to prepare an MCB right away and test it rather than doing extensive testing of earlier stages. The results of early testing have only secondary relevance over MCB, WCB, EOP testing.	Cell Seed as **optional or consider omitting these columns.**

		Initial cell evaluation: Only culture parameters (viability, growth) feasible at this stage of development. ID, sterility (bioburden), stability, microbial agent, tumorigenicity) will not typically be done until cells are banked. Cell seed: Cytogenetic: For CCL, karyotypic analysis is not generally useful.	
	Table 4: MCB, WCB, EOP	General: Microbial agents: Breakdown of type of tests recommended would be useful Could be done in separate table	Breakdown types of microbial agents testing.
		General: for virus testing, a table analogous to Table 1 of ICH Q5A would be helpful.	For virus testing, include a table analogous to Table 1 of ICH Q5A
		MCB: Cytogenetic: For CCL, karyotypic analysis is not generally useful. Homogenity: stability and integrity of cryopreserved vials is demonstrated when vials are thawed for production and demonstrated to produce intended product at scale. Also applies to WCB.	Note that for CCL karyotypic analysis is not generally useful.
		WCB: Cytogenetics: See	Reference abbreviated testing

		comments made for MCB. Microbial agents: Not necessary to repeat the extensive characterization performed on MCB for each WCB.	for WCBs derived from extensively characterized MCBs.
		<u>EOP:</u> Add (+) for sterility and identity to the End of Production (EOP) column Stability: Genetic characterization is done one time only at maximum in vitro cell age intended for production. This analysis compares EOP to MCB. A sponsor may choose to evaluate MCB in some tests earlier in development to mitigate risk. Cytogenetics: See comment for MCB.	Add (+) for sterility and identity in the EOP column.
	141/3119 footnote	The intent of the parentheses is not clear	Clarify the intent of the parentheses.
Part C. Risk reduction strategies during the manufacture of biologicals from animal cell substrates	142	Related to risk mitigation strategies, it would be valuable to add the recommendation to consider pre-treatment of the bioreactor feed (medium components etc.) to minimize access of	

		viruses to the bioreactor.	
	142/3138 Examples of exogenous agents introducted during manufacturing.	There is an additional example virus contamination that came from a QC testing lab that was published as an abstract in *Biologicals.* In this case it was due to an environmental contamination due to mouse presence in the testing lab. The text of the abstract is copied to the right and the reference from Barbara Potts is as follows: ***Biologicals XX 2009 pages 1-10. James S. Robertson et al are the authors. Title Meeting Report Virus & TSE safety forum 2008***	**Additional Example:** C.2.1.4 Example: Bluetongue virus contamination because of uncontrolled environment during QC Testing ***"Characterization of a Viral Cell Culture Contamination"*** was discussed by Barbara Potts (Genentech). Dr. Pott's talk presented the Genentech perspective of the Bluetongue virus lab contamination event described by Rangarajan Sampath. Based on TEM analysis, the mystery virus was initially believed to possess an envelope, which misdirected the investigation for some time. After several unsuccessful attempts to narrow down enveloped viruses that fit the infection pattern of the mystery virus, genetic analyses were employed. They included restriction enzyme-defined sequence independent single primer amplification performed by Virginia Polytechnic Institute and identification by the Ibis T5000 system. Both

			analyses identified the mystery virus as Bluetongue virus. Bluetongue virus infects cattle and is transmitted by midge bites. This contamination was not definitively traced, but was surmised by Genentech to be introduced to the lab via infected rodents, rather than bovine products.
C.1 General considerations			
C.2 Risks			
Appendix Tumorigenicity protocol using athymic nude mice to assess mammalian cells			