



GlaxoSmithKline

December 21, 2006

Management Dockets, N/A
Dockets Management Branch
Food and Drug Administration
HFA-305, Room 1-23
12420 Parklawn Dr
Rockville, MD 20857

GlaxoSmithKline
PO Box 13398
Five Moore Drive
Research Triangle Park
North Carolina 27709-3398
Tel. 919 483 2100
www.gsk.com

Re: NAS 0; Not Product Specific

General Correspondence: Comments on Draft Guidance for Industry: "Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the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" [Docket No. 2006D-0383]

Dear Sir or Madam:

Reference is made to the notice published by FDA in the Federal Register on September 29, 2006 to invite written comments on a new draft guidance for industry, "Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the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." The purpose of this submission is to provide comments from GlaxoSmithKline on this draft guidance.

GSK is a research-based pharmaceutical and biotechnology company. Our company is dedicated to the discovery, development, manufacture and distribution of medicines and vaccines that enable people to lead longer, healthier and more productive lives. We appreciate the opportunity to comment on this draft guidance and provide input into your efforts to update the 1993 document entitled, "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals", as well as the ICH Guidances Q5A and Q5D.

We have the following general comments, which are followed by specific comments that include references to the applicable sections in the draft guidance. We have designated our key comments as *Critical* or *Major*, to denote specific issues that must be addressed in the final guidance, and our recommendations for edits are provided.

General Comments

- GSK feels it would be appropriate for FDA to specify its requirements and expectations for mycoplasma testing in a separate guidance so that information

can be easily identified (versus only included in copious text of various guidance documents).

- The document often does not make a distinction between tests / study designs for which the intended test articles are cell banks or cells used in production and tests/study designs for which the intended test articles are harvest or bulk materials. For any given stage of manufacturing, this lack of clarity can lead manufacturers to perform testing that is not scientifically justifiable, which in turn can result in delays in development and manufacturing, as well as an inappropriate data package for FDA review. Distinctions should be made in the description of each stage with respect to which testing is required and to which products the tests apply. Integration of specific examples would also be helpful in this regard.
- Additionally, the document does not appear to make a distinction between testing required for recombinant subunit vaccines, which are highly purified and included in the ICH documents, and vaccines which are excluded from the scope statements for these documents. This apparent lack can lead to some confusion on the part of manufacturers who wish to submit regulatory filings both within and outside the United States.
- The term “might” is used consistently throughout the draft (106 times) which seems an unprecedented usage for what can be an ambiguous term. While the industry acknowledges and fully appreciates that this is a guidance document and therefore by nature is written to allow the sponsor a certain level of flexibility in approach (based on evolving science and technology), additional detail regarding specific FDA expectations and requirements, where applicable would be appreciated. In this regard, rather than using the word “might” in all cases, it would be helpful to have additional examples of situations where sponsors should consider particular testing using specific materials or methodology(s) at various manufacturing and testing points.
- The scope statement for ICH Q5A specifically excludes inactivated vaccines and all live vaccines containing self-replicating agents; only recombinant subunit vaccines are included.

In general, it is apparent that there are instances of inconsistency between this Draft Guidance and previous documents that have been issued since the 1993 Points to Consider, such as ICH Q5A, Q2A and Q5D. In the spirit of harmonization, FDA should reconcile the information in this Draft Guidance with those documents where applicable.

Furthermore, as this document only addresses starting materials for viral vaccines, GSK requests an update of the 1993 Points to Consider to focus on products that were included in the 1993 document but are outside the scopes of both the ICH documents and this new guidance.

- The scope of the draft guidance, limited to cell substrates of human or animal origin, covers some less classical cellular substrates such as insect cell lines.

Specific Comments

Section II OVERVIEW: CHARACTERIZATION AND QUALIFICATION OF CELL SUBSTRATES

1. §II.A. (**Background**, Paragraph 2, 3rd sentence). *Major*

The term, “potential oncogenic agents”, in this sentence is too broad. The agency may want to specify the agents as virus here. We suggest that the sentence be reworded to read:

“...and potential oncogenic viruses.”

2. §II.A. (**Background**, Paragraph 4, last sentence). *Major*

The last sentence states, “In some situations, additional validation studies to demonstrate...” We consider this a misnomer, in that actual clearance studies are not validated, the readout assays are. Recommend the sentence be revised as:

“In some situations, additional studies to demonstrate...”

3. §II.B.1 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates: **Vaccine Purity**, Paragraph 1, last sentence). *Major*

The guidance states that “...viral vaccines to validate clearance of any adventitious agent.” We believe that this is a challenge of process capabilities, not a validation exercise and the sentence should be revised to reflect the correct exercise. Suggest wording:

“...viral vaccines to demonstrate clearance of adventitious agents.”

4. §II.B.1 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates: **Vaccine Purity**, Paragraph 2, first sentence). *Major*

The draft guidance reference the ICH Q5A (Ref. 2) in this sentence "...more reliance on process validation (Ref. 2)." ICH Q5A does not discuss process validation, nor does it infer that a determination of viral clearance is part of process validation. Initial studies performed to support the use of the product in phase 1 clinical trial use materials from processes that are not yet validated.

We recommend that the references to validation throughout this section be rewritten to reflect process challenge and process capabilities (see comments previous and just following this comment). Suggest that this sentence be re-worded to read:

"...more reliance on the clearance capacity of the manufacturing process (Ref. 2)."

5. §II.B.1 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates: **Vaccine Purity**, Paragraph 2, third sentence). *Major*

The draft guidance indicates the validation of adventitious agents inactivation "...provide documentation of your validation for inactivation of adventitious agents." This is a challenge of process potential, not a validation exercise. Suggested wording:

"...provide data to support your claim for inactivation ..."

6. §II.B.1 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates: **Vaccine Purity**, Paragraph 3, first sentence). *Minor*

Edit the first sentence in the third paragraph to read: **"...vaccine, including those used to treat the starting materials, as the..."**

7. §II.B.1 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates: **Vaccine Purity**, Paragraph 3, last sentence). *Major*

The draft guidance states in the last sentence that "Certificates of Analysis (COA) for all reagents and biological raw materials used for vaccine production should be

included in your submission.” We suggest that limiting only COAs for biological raw materials which are critical should be provided; all other material COAs should be available on inspection. Revised wording:

“Certificates of Analysis (COA) biological raw materials used for vaccine production should be included in your submission.”

8. §II.B.1 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates: **Vaccine Purity**, Paragraph 4, first sentence). *Minor*

Edit the first sentence to include end point dilution as an efficient technique for cloning. Suggested wording:

“... (e.g., by molecular cloning, serial passage or cloning using end-point dilution ...).”

9. §II.B.2 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates : **Potential Sources of Contamination**, Paragraph 1, last sentence) *Major*

The “Testing contaminating agents.....” stated here is too broad a term. The testing should be limited to the relevant agents that are known pathogens for humans and that could be found as contaminants given the passage history. We recommend the sentence be revised. Suggested wording:

“Testing might be needed to verify the absence of additional contaminating agents, particularly those agents that are human pathogens whose propagation given their passage history might be supported by your cell substrate.”

10. §II.B.3 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates : **Quality Design**, Paragraph 1, 1st & 2nd sentences) *Minor*

The draft guidance references to 21 CFR Part 58. This should be deleted since the GLP regulations are specific for safety testing.

11. §II.B.4 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates : **Use of Control-Cell Cultures**, Paragraph 1, 3rd sentence) *Editorial*

Edit the sentence for clarity. Suggested wording:

“...presence of adventitious agents by direct observation, and testing of the cell sheet and...”

12. §II.B.4 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates : **Use of Control-Cell Cultures**, Paragraphs 1 & 2) *Comment*

The Agency should clarify the use of control-cell-culture stated in these paragraphs: “(¶1) If you are using primary cell culture to propagate your virus...In this situation, you should produce and test uninfected control-cell cultures...(¶2)Use of control-cell cultures is important when your vaccine might interfere with results of in-process testing of the product; for example when the virus cannot easily be neutralized to permit testing for adventitious agents.”

It appears that control cells are only required when primary cell cultures are used for production or when the product interferes in the test system and cannot easily be neutralized to enable testing for extraneous agents. This should be further clarified in the text, accordingly.

13. §II.B.4 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates : **Use of Control-Cell Cultures**, Paragraph 2, 2nd sentence) *Major*

The draft guidance states “You should produce and test uninfected control-cell cultures that are derived in parallel with and handled in the same manner as the production culture.” In some instances, control cells cannot be handled exactly in the same way as production culture as indicated in this sentence (see examples in next comment). We suggest that the sentence be revised to allow for flexibility in handling of control-cell cultures. Revised wording:

“You should produce and test uninfected control-cell cultures that are derived in parallel with and handled in the same manner whenever and wherever possible as the production culture. Alternative culture conditions may be implemented if justified.”

14. §II.B.4 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates : **Use of Control-Cell Cultures**, Paragraph 2, 3rd sentence)

Critical

The draft guidance states “You should use a culture period of at least 14 days...” We recommend the sentence be revised to allow for proper handling of different cell cultures. For example, for some cells cultured in suspension (e.g. Hi-5, CHO), it is impossible to maintain the cells for long periods of time without subculture.

Therefore, by default, the handling of the cells will not be identical to that applied for the production cells. We recommend the sentence be revised to:

“You should use a culture period longer than the period used for the production of the viral harvest and, if applicable, at least 14 days. Alternative periods (because of the cell nature) may be appropriate.”

15. §II.B.4 (Product-Specific Parameters Influencing Characterization and Qualification of Cell Substrates : **Use of Control-Cell Cultures**, Paragraph 2, 4th sentence)

Minor

The requirement for testing of control cells and end-of-production cells indicated in this sentence “Testing of control cells does not always eliminate the need for testing end-of-production cells, which might be required to demonstrate the absence of agents induced during vaccine manufacture” is not clear. The agency should provide clarity on the normal/appropriate time during product development for testing of EOPC and allow flexibility to not test routinely (per other guidance). Please also provide an example of circumstances under which a sponsor would be required to test the EOPC in production. Suggested wording:

“Testing of control cells does not always eliminate the need for testing end-of-production cells, which might be required to demonstrate the absence of agents induced during vaccine manufacture. These end-of-production cells might be tested during the validation of the MCB or the WCB.”

Section III. CHARACTERIZATION AND QUALIFICATION OF CELL SUBSTRATES, VIRAL SEEDS, BIOLOGICAL RAW MATERIALS AND VACCINE PRODUCTION

16. §III.A.1 (Properties of the Cell Substrate : **Properties relevant to cell substrate selection**, Paragraph 3, 1st sentence)

Major

The requirement for providing information for starting materials stated in the sentence “Whatever starting materials are used for generation of the cell substrate...” is too broad. Recommend that the requirement be limited to the starting materials that contribute to the generation of the cell substrate. Revised wording:

“For each starting material (e.g. cells, plasmids) that contributes directly to the generation of the cell substrate, complete information including characterization should be provided.”

17. §III.A.1 (Properties of the Cell Substrate : **Properties relevant to cell substrate selection**, Paragraph 3, last sentence) *Editorial*

Edit the sentence “See Sections III.A.2. for on donor screening.” to read “**See Sections III.A.2. through III.A.7. for additional information.**”

18. §III.A.2 (Properties of the Cell Substrate : **Source**, Paragraph 1, 6th sentence) *Editorial*

Edit ‘Issues ... are discussed in ...’ to read “**Issues ... discussed in ...**”

19. §III.A.3 (Properties of the Cell Substrate : **History and other important characteristics**, Paragraph 1, 1st sentence) *Minor*

Edit the “.. adherence to GLPs or cGMPs ..” to read “**..adherence to cGMPs ..**” The GLP regulations are specific for safety testing.

20. §III.A.3 (Properties of the Cell Substrate : **History and other important characteristics**, Paragraph 1, 1st & 3rd sentences) *Minor*

The requirements stated in the draft guidance “(¶1)..listing of any other agents grown in the facilities around the time of cell substrate passage"....."(¶3)You should provide documentation of all raw materials you used for the entire passage history” should be narrowed to the production unit (more relevant than larger facility). In some instances, the level of documented historical detail may be limited; therefore sponsor should be required to provide as much information as is practically available. Should be considered that certain Cell Banks or certain Virus Seeds are developed by parties other than the sponsor, e.g. in University laboratories.

Suggested wording:

“listing if available of any other agents grown in the production unit around the time of cell substrate passage”.....”You should provide all documentation available for all raw materials from human or animal origin that you used for the entire passage history”

21. §III.A.3 (Properties of the Cell Substrate : **History and other important characteristics**, Paragraph 3) *Minor*

This requirement needs to be revised since medical history of the donor is not always available; acknowledgement of potential inability to provide comprehensive medical information on the donor (and therefore to supplement with other information) is also in harmony with requirements of ICH.

Suggested wording:

**“You should also provide the following:
donor's medical history and results of tests performed on the donor for the detection of adventitious agents...introduced into the cell substrate. For instances in which the specified information is not available (eg. donor medical history), data derived from analysis of the substrate by other methods may prove supportive and may be required.**

22. §III.A.3 (Properties of the Cell Substrate : **Growth Characteristics**, Paragraph 1, 3rd and 4th sentences) *Major*

The requirement stated here should be consistent with the Draft Guidance Section *III.B.4. Special consideration for diploid cells* where it is mentioned that animal tumorigenicity testing is not needed if you are using genetically unmodified diploid cell strains such as MRC-5 and WI-38 and FRhl-2, because their extensive previous characterization and well-defined non-tumorigenic phenotype satisfies the requirement in 21 CFR 610.18.

Suggested wording:

“Per 21 CFR 610.18(C)(1)(ii), a description of the tumorigenic property of the cells is required for all diploid and non-diploid cells. However, the requirements in this regulation are not applicable to diploid cell strains that are not genetically modified and are not novel, such as MRC-5, WI-38 and FRhl-2, as they are extensively characterized and well-defined, and their non-tumorigenic phenotype satisfies these CFR requirements (see also section III.B.4 of this guidance)...”

23. §III.A.5 (Properties of the Cell Substrate : **Expression Characteristics** , Paragraph 1, 3rd sentences) *Comment*

For characterization of expression, the draft guidance states “In some cases...to evaluate expression of other genes relevant for cell phenotype.” It would be helpful to have specific examples of when this should be performed?

24. §III.A.5 (Properties of the Cell Substrate : **Expression Characteristics** , Paragraph 1, last sentences) *Comment*

The draft guidance states “If viral sequences are related to the expression system, you might need to assess their infectivity and potential interference with adventitious agents testing.” Please define what is meant by viral sequence.

25. §III.A.6 (Properties of the Cell Substrate : Susceptibility to adventitious agents, Paragraph 1, 2nd sentence) *Major*

The requirement for testing each lot noted in the sentence: “....specific tests were required to assay for these viruses in each lot .;” is not practical or feasible. Testing for all possible contaminants on a routine lot-to-lot basis; therefore lot-to-lot testing

should be implemented, as necessary, based on agents identified during characterization of the cell substrate.

Suggested wording:

“If viruses were detected in the cells used for production, lot to lot testing should be put in place.”

26. §III.A.7 (Properties of the Cell Substrate : **Generation of Cell Substrates**, Paragraph 1, 4th sentence) *Comment*

For characterization of cell substrate, the draft guidance notes “In addition, a cell substrate that has been derived by cell cloning might have different characteristics from the parenteral cell line. Because it is derived from one or a few cells, it might not have characteristics representative of the original population from which it was cloned.”

It should be clarified if a well-characterized cell line that is grown in a new culture medium is considered as a new cellular substrate that needs full characterization

27. §III.B.1 (Cell Banking : **Cell Banking Strategies and Methods**, Paragraphs 1, 2, & glossary) *Minor*

We noted that the definitions for the Master Cell Bank and the Working Cell Bank in the draft guidance are inconsistent with those in ICH Q5A / Q5D. The MWCB does not exist in ICH documents.

28. §III.B.1 (Cell Banking : **Cell Banking Strategies and Methods**, Paragraph 4, 2nd sentence) *Minor*

We consider this instruction is too open-ended to be meaningful without inclusion of a rationale for the choice of test point. Suggest the sentence be revised to read:

“.. should be completely characterized and the choice of that test point should be justified.”

29. §III.B.2 (Cell Banking : **Qualification of Cell Banks and Primary Cells,**
Paragraph 2) *Major*

Other than a filtration based test for bacteria and fungi, it is not feasible to perform tests for mycoplasma or viruses on cells directly from the cell bank ampoules for at least three reasons: (1) the cryoprotectant in the freeze medium will interfere with a number of the tests; (2) the tests should be performed on cells in their culture media; and (3) too many ampoules of the MCB would need to be used to complete the testing

Suggested wording: “Testing to qualify the MCB should be performed directly on the cell bank, except when it is more appropriate to test cell cultures derived from the cell bank or when the MCB amounts are too limited.”

30. §III.B.2 (Cell Banking : **Qualification of Cell Banks and Primary Cells,**
Paragraph 4, 1st sentence) *Minor*

The sentence “Either the MCB or all animal-derived reagents ..” implies that complete testing of reagents can substitute for some of the testing on the MCB, and does not take into account the potential for amplification of low level contaminants while expanding the culture to generate sufficient cells for banking. We suggest rewording the sentence to read:

“The MCB and all animal-derived reagents to which it has been exposed should be shown.”

31. §III.B.4 (Cell Banking : **Special Considerations for Diploid Cell Strains,**
Paragraph 2) *Major*

The full karyotype should not be required for well-characterized cell lines such as WI-38, MRC-5, and FRhL2 ... when these cell lines have not been modified genetically.

32. §III.B.5 (Cell Banking : **Special Considerations for Continuous Cell Lines and §7 End -of Production Cells,** Paragraph 4, 1st sentence) *Editorial*

We suggest using ICH terminology for ‘end of production’ and ‘EOPC’ for the consistency with internationally accepted terminology.

33. §III.B.7 (Cell Banking : **End -of-Production Cells**, Paragraph 1, 1st sentence)
Minor

Edit the sentence “Your characterization should include...stability of expression of the inserted or engineered genes and genetic stability” to add “if applicable” at the end of the sentence as this should be applicable for genetically modified cell substrates.
Suggested wording:

“Your characterization should include...stability of expression of the inserted or engineered genes and genetic stability, if applicable”

34. §III.C. (Viral Seeds, Paragraph 2, 2nd sentence) *Major*

The viral seeds storage condition noted in the draft guidance are too limited. The working seeds can also be stored at -70°C. We recommend that the sentence be revised as:

“Viral seeds should be stored in liquid nitrogen or at -70°C and in more than one location....”

35. §III.C.1 (Viral Seeds : **Master Viral Seed**, Paragraph 2, 1st sentence)
Minor

It should be clarified in which circumstances the identity of the virus seed lots requires sequencing of the entire genome in case of live attenuated virus.
Recommend the sentence be revised for clarity as suggested below:

“You should perform tests for identity (which could necessitate sequencing the entire vaccine virus or the relevant part of the live attenuated vaccine virus.”

36. §III.C.1 (Viral Seeds : **Master Viral Seed**, Paragraph 4, 1st sentence)
Major

We recommend that the term “often” used in the sentence be changed to “might”. This is an instance where the use of the term “might” or “may” provides the sponsor with appropriate flexibility to accommodate current science.

In recent discussions in the scientific community it was suggested that the potential neurovirulence of the vaccine strain should rather be considered during preclinical

development, based on available data, notably for wild type virus or based on results from test carried out on the vaccine strain using an animal model that differentiates wild type and attenuated virus. The requirements for neurovirulence testing of the Working Seeds were reviewed at a joint EDQM-WHO-IABS scientific workshop. Ph. Eur. Monographs for all live attenuated vaccines were reviewed according to the conclusions of this meeting. Except for the oral poliomyelitis vaccine, the routine test of neurovirulence for all the other live attenuated Virus Seeds will be suppressed in the Ph. Eur.

37. §III.C.2 (Viral Seeds : **Working Viral Seed** , Paragraph 1, 1st sentence) *Major*

The draft guidance suggests that “You may subject the Working Virus Seeds (WVSs) to less rigorous characterization than the MVSs from which they were derived.” Like for the Cell Bank extensive testing should be allowed on the Master Viral Seeds or on the Working Viral Seeds given the limited amount of Master Viral Seeds. The testing of the MVS will be a one-time testing. We recommend that this sentence be revised to:

“You may subject the Working Virus Seeds (WVSs) to less rigorous characterization than the MVSs from which they were derived. Alternatively, some manufacturers may choose to extensively characterize each WVS in lieu of thorough characterization of the MCB.”

38. §III.D (Biological Raw Materials and ancillary Reagents, Paragraph 2, 3rd sentence) *Editorial*

Edit the sentence to include sections referenced in Sections D1 through D4.
Suggested wording:

“...are discussed below and in Section IV.”

39. §III.E.2 (Considerations in Testing at Different Stages of Production : **Pre-production cells**, Paragraph 1) *Minor*

Edit the sentence to read “Pre-production cells: an identity may be performed on cells used for production.”

40. §III.E.3 (Considerations in Testing at Different Stages of Production : **Pre-Filtered Harvest or End-of-Production Cells**, Paragraph 2, 1st sentence)

Critical

The draft guidance states “In addition to testing the viral or vaccine bulk for cultivatable mycoplasma . . .and adventitious viruses by in vitro and in vivo methods.” Testing for adventitious viruses by in-vivo methods is not necessary at this stage. Potential adventitious viruses have been tested by in-vivo methods on the cell banks and viral seeds.

The purpose of testing the downstream manufacturing stages is to assess any potential for contamination that may have occurred during the manufacturing process (and therefore, adherence to GMPs). This can be appropriately and specifically accomplished by employing the in vitro viral screening method alone. The utility of the burdensome in vivo method at this juncture in the process is questionable.

Typo – add “t” to “he”

We recommend that the sentence be revised to:

“In addition to testing the viral or vaccine bulk for cultivatable mycoplasma....and adventitious viruses by in vitro methods.”

41. §III.E.3 (Considerations in Testing at Different Stages of Production : **Pre-Filtered Harvest or End-of-Production Cells**, Paragraph 4, last sentence) ***Minor***

Edit the sentence to read: “If multiple harvests are performed for a single vaccine lot, testing may need to be performed on each individual harvest in order to avoid dilution of a potentially contaminated harvest with uncontaminated harvests. For example, this may be relevant when the test method used has a low sensitivity”

42. §III.E.5 (Considerations in Testing at Different Stages of Production : **Post-Filtered Harvest or Final Bulk**, Paragraph 1, 4th sentence) ***Major***

The draft guidance states: “These include testing for levels of residual cellular proteins and cellular nucleic acids.” We recommend that the term “may” be included in this sentence to allow flexibility in the need for routine testing as it may be possible to omit these tests from routine testing if the manufacturing process is validated to consistently achieve the specification.

Additionally, this will align with WHO Guidelines to assure the quality, safety and efficacy of live attenuated Rota virus (oral).

Suggested wording:

“These may include testing for levels of residual cellular proteins and cellular nucleic acids.”

Section IV DESCRIPTION OF QUALITY-CONTROL TEST METHODS

43. §IV.A (Testing of Adventitious Agents, Paragraph 1, 1st sentence) *Minor*

The stated testing of adventitious agents “Your biological starting materials should be characterized to ensure that they are free from extraneous infectious organisms such as bacteria, fungi, cultivatable and non-cultivable mycoplasmas and spiroplasma, mycobacteria, viruses...” does not provide industries with flexibility. Depending of the source (country, organ) of the raw materials, certain tests noted here are not relevant. We suggest the sentence be reworded to read:

“Your biological starting materials should be characterized, if appropriate, to ensure that they are free from extraneous infectious organisms such as bacteria, fungi, cultivable and non-cultivable mycoplasmas and spiroplasma, mycobacteria, viruses...In developing a characterization plan, consideration should be given to factors such as country of origin of the materials, tissue type, etc.”

44. §IV.A (Testing of Adventitious Agents, Paragraph 2, 3rd sentence) *Major*

The Agency needs to provide additional clarity/strength of tests, use of alternatives such as those recommended by the WHO or the EP should be justified.

45. §IV.A.1 (Testing of Adventitious Agents : **In-vivo Tests**, Paragraph 1, 1st sentence) *Major*

We recommend that the use of embryonated eggs for the testing of virus seed noted in sentence be removed in order to align with Ph Eur. and WHO. Suggested wording:

“In the development of viral vaccines...and suckling mice.”

46. §IV.A.1.e (Testing of Adventitious Agents : In-vivo Tests : **Embryonated Chicken Eggs**, Paragraph 2, 4th sentence) **Major**

The draft guidance states “Both the initial pool and the passaged harvest should be tested for the presence of hemagglutinating agents with red cells from guinea pigs, humans (type O) and an avian species”

The routine manipulation of human red blood cells is of increasing concern from a personnel safety perspective. The proposal is to keep only guinea pig red blood cells. A broader spectrum of relevant red blood cells should be used for extensive characterization of Cell Banks and Seeds.

Suggested wording:

“Both the initial pool and the passaged harvest should be tested for the presence of hemagglutinating agents with red cells e.g. from guinea pigs, the animal source being chosen based on the passage history.”

47. §IV.A.1.f (Testing of Adventitious Agents : In-vivo Tests : **Antibody Production Tests**, Paragraph 2, 1s sentence) **Minor**

The antibody production test described in the draft guidance “A specific in vivo test for LCMV ...when specific concerns about LCMV exist (ie. Antibody detected)...” is not clear. If no antibody against LCMV detected, can we conclude that there is no concern, therefore no requirement?

48. §IV.A.2.a.ii (Testing of Adventitious Agents : In Vitro Tests for Viruses : Monkey kidney cells : **Methods**, Paragraph 1, 3rd & 4th sentences) **Major**

The Agency should provide clarity to the in-vitro test method described in this sentence: “The cell cultures should be observed for at least two weeks. After two weeks of observation, supernatants or lysates are subcultured onto fresh cells and observed for at least an additional two weeks.”

It is unclear from the text to what stage of the manufacturing process the document is referring (ie. Cell culture or harvest). Assuming the document is referring to the harvest stage, the (14d + 14d) requirement specified differs from the revoked 21 CFR Part 630 Additional Standards and the requirement of the Ph. Eur., and from the test described in the WHO TRS. The additional 14 days will have as significant impact

on the turn-around time for testing and the capacity/throughput capabilities of most quality laboratories.

We suggest the following wording:

“The cell cultures should be observed for at least two weeks. Based on the passage history and if a contamination is suspected, supernatants or lysates are subcultured onto fresh cells and observed for at least an additional two weeks if appropriate.”

49. §IV.A.2.a.ii (Testing of Adventitious Agents : In Vitro Tests for Viruses : Monkey kidney cells : **Methods**, Paragraph 3, 4th sentences) *Major*

The draft guidance notes that “The test for haemadsorbing and hemagglutinating viruses is generally performed at the end of the observation period using guinea pigs, chicken and human type O RBCs...” We recommend that the sentence be revised to align with the Ph. Eur. Paragraph 2.6.16 that requires only guinea pigs RBCs,

Suggested wording:

“The test for hemadsorbing and hemagglutinating viruses is generally performed at the end of the observation period using guinea pigs RBCs.”

50. §IV.A.2.a.ii (Testing of Adventitious Agents : In Vitro Tests for Viruses : Monkey kidney cells : **Methods**, last paragraph) *Minor*

As this test can detect compromise by an adventitious virus during manufacturing, substituting the control cells for the production cells can yield meaningful data only if the control cells are handled in an identical manner as the production cells

51. §IV.A.2.c (Testing of Adventitious Agents : In Vitro Tests for Viruses : **Biochemical tests for retroviruses**, Paragraph 2, 2nd sentence) *Minor*

The draft guidance notes “The lower limit of detection should be comparable with the published literature (ref. Arnold et al., 1998).” In the abstract the assay is described

as 1 million fold more sensitive than conventional assays. The exact lower limit of detection should be noted. Suggested wording:

“The lower limit of detection should be comparable with the published literature (i.e. at least xxxx)”.

52. §IV.A.2.c (Testing of Adventitious Agents : In Vitro Tests for Viruses : **Biochemical Tests for Retroviruses**, Paragraph 3, 2nd sentence) *Minor*

Provisions for demonstrated consistency should be added; as is the case for manufacture of flu vaccines. We suggest the sentence be revised to read:

“For example, products manufactured from primary cells might need to be assessed lot-by-lot unless proven consistency has been demonstrated.”

53. §IV.A.2.c & d (Testing of Adventitious Agents : In Vitro Tests for Viruses : **Biochemical Tests for Retroviruses & Infectivity Test for Retroviruses**) *Minor*

Retroviruses are endogenous sequences in the production substrate, rather than adventitious contaminants. Suggest that these 2 sections be placed into a separate section.

54. §IV.A.2.d (Testing of Adventitious Agents : In Vitro Tests for Viruses : **Infectivity Test for Retroviruses**) *Minor*

The draft guidance recommended that “For non-murine retroviruses, infectivity testing on appropriate indicator cells (selected for their susceptibility to different retroviruses types)...”

This recommendation on what cell substrate to be used is not clear and should be specified.

For human vaccine, the main reason for testing relates to human infectivity. Hence human cell substrate should be used (e.g. 293 cells). Suggested wording:

“For non-murine retroviruses, infectivity testing on appropriate indicator cells (selected for their susceptibility to different retroviruses able to infect humans, e.g., 293 cells)....”

55. §IV.B (Testing of Cell Properties)

Minor

There is no test description for identity test of cell substrate.

56. §IV.B.1 (Testing of Cell Properties : **Tests for Tumorigenicity**,
Paragraph 8, 2nd sentence)

Major

The test duration for tumorigenicity stated in the sentence “Weakly tumorigenic cells might require between 4 and 7 months to form tumors in nude mice” should be reduced to three months in order to align with Ph Eur and WHO for 84 days.
Suggested wording:

“Weakly tumorigenic cells might require up to 3 months to form tumors in nude mice.”

57. §IV.B.2 (Testing of Cell Properties : **Tests for Oncogenicity**)
Comments

The requirement for testing cells for oncogenic agents stated in the draft guidance is not clear. It is important for the Agency to clarify whether oncogenicity study is needed for a non-tumorigenic cell line.

58. §IV.B.2 (Testing of Cell Properties : **Tests for Oncogenicity**,
Paragraph 1, 3rd sentence)

Major

The draft guidance states “If your vaccine is manufactured in a cell substrate that was derived from a tumor or that has developed a tumorigenic phenotype through an unknown mechanism, it might carry a higher theoretical risk of containing oncogenic substance.”

The test should only be required for cell lines with tumorigenic potential or derived from tumors. Suggested wording:

“If your vaccine is manufactured in a cell substrate that was derived from a tumor or that has a tumorigenic phenotype through an unknown mechanism, it might carry a higher theoretical risk of containing oncogenic substance.”

59. §IV.B.4 (Testing of Cell Properties : **Testing for Genetic Stability**, Paragraph 1, 3rd sentence) *Editorial*

Edit the sentence to read: “...**be expressed at comparable levels ..**”

60. §IV.B.4 (Testing of Cell Properties : **Testing for Genetic Stability**, Paragraph 1, 6th sentence) *Minor*

Relevance of reference to Q2B (methods validation) is not clear.

61. §IV.C.2 (Other Tests: **Testing for Residual DNA**, Paragraph 3, 2nd sentence) *Major*

The draft guidance notes “For widely used human diploid cell strains, such as MRC-5 and WI-38, measurement of residual DNA might be unnecessary”

FRhL-2 cells are also well-characterized diploid cells. We recommend adding this cell lines to be consistent with paragraph on tumorigenicity. Suggested wording

“ For widely used human diploid cell strains, such as MRC-5, WI-38 and FRhL-2 cells measurement of residual DNA might be unnecessary”

62. §IV.C.2 (Other Tests: **Testing for Residual DNA**, Paragraph 3, 4th sentence) *Major*

The draft guidance states “You should limit residual DNA for continuous non-tumorigenic cells, such as low-passage Vero cells, to less than 10 ng/dose for parenteral inoculation as recommended by WHO.”

Reference should be made to the WHO “Guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccine” for where the an acceptable limit of not more than 100µg of cellular DNA per human dose is likely to provide an adequate margin of safety for orally-delivered vaccines. We suggest the sentence be reworded to:

“You should limit residual DNA for continuous non-tumorigenic cells, such as low-passage Vero cells, to less than 10 ng/dose for parenteral inoculation and to less than 100 µg/dose for oral vaccine as recommended by WHO.”

63. Glossary : Item 22 *Minor*
Please include definition

64. Glossary : Item 33, 2nd sentence *Minor*

Please clarify: "...demonstration of what characteristics the process is capable of performing .."

65. Section VII: Reference List *Minor*

The reference listed the listed ICH Guideline Q2A. As of Nov, 2005, ICH Guideline Q2A was replaced by Q2(R1). A search for Q2A on both the CBER and ICH websites results in no Q2A document. However, FDA has not notified the public of new Q2(R1) in Federal Register Ref. 6.

APPENDIX 1: Table 1. Example of a Testing Scheme for Manufacture of a Viral Vaccine

66. Table 1: Virus Seed *Major*

- Listed testing of "Mycoplasma/Spiroplasma" on viral seed should be change to **"Spiroplasma testing required if insect origin. Spiroplasma if appropriate"**
- Listed testing of "Identity, /potency/Activity /, infectious titer" on viral seed should be removed. These tests are performed on harveststep final.

1. Table 1: Control cell cultures *Major*

The listed testing of "Spiroplasma / *in vivo* adventitious agents / bovine and porcine viruses /BK /Specific agents" using control cell cultures should be removed. These tests are performed on viral harvest.

2. Table 1: Master cell bank *Minor*

- Spiroplasma testing is required if insect origin. Suggest: **"- spiroplasma (if applicable)."**
- Revise "tumorigenicity (except rodent cell lines)". Suggested wording: **"tumorigenicity (except rodent cell lines and tumorigenic cell lines)"**

1. Table 1: Vaccine bulk *Minor*
 - The listed testing of “spiroplasma / *in vivo* adventitious agents” should be removed at this step. These tests are done on seed and cell bank.
 - Revised the “RT assay” at this step to: “**RT assay (if applicable)**”

1. Table 1: Final filled product *Minor*

The “Spiroplasma” testing should be removed. This test is done on seed and cell bank if applicable. “

Again, we thank you for the opportunity to provide comments. This submission is provided in electronic format according to the instructions provided at <http://accessdata.fda.gov/scripts/oc/dockets/commentdocket.cgm?AGENCY=FDA>.

Please contact me at (919) 483-6405 if you require clarification or have any questions about these comments. Thank you.

Sincerely,



Anne N. Stokley, M.S.P.H.
Senior Director, Policy, Intelligence & Education
US Regulatory Affairs