

22 December 2006

Division of Dockets Management (HFA-305)  
Food and Drug Administration  
5630 Fishers Lane, Room 1061  
Rockville, MD 20852

**Re: Docket No. 2006D-0383:** Draft Guidance for Industry on Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines for the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 [71 Federal Register 57547, September 29, 2006]

Dear Sir/Madam,

Sanofi Pasteur Inc. of Swiftwater, Pennsylvania thanks the Food and Drug Administration (FDA) for the opportunity to comment on the above-referenced draft guidance for industry entitled, "Characterization and Qualification of Cell Substrates and Other Biological Starting Materials Used in the Production of Viral Vaccines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." Headquartered in Lyon, France, sanofi pasteur is the vaccines business of sanofi-aventis Group. Sanofi-aventis is the world's third-largest pharmaceutical company.

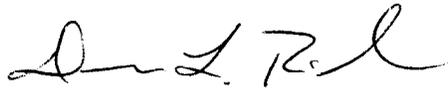
Sanofi pasteur is a world leader in vaccines and produces more than one billion doses of vaccines every year to immunize over 500 million people around the world. Sanofi pasteur, in close consultation with the US public health establishment, including the FDA, and Centers for Disease Control and Prevention (CDC), strives to alleviate the suffering and death resulting from vaccine-preventable diseases.

We appreciate this draft guidance and believe that its valuable content will be useful for the manufacture of viral vaccines under its scope. Comments are provided in the attached table and indicate points where clarification would be helpful. We would also like to provide general comments regarding harmonization. As a global manufacturer, sanofi pasteur must consider requirements from different geographic regions. Thus, we would appreciate international harmonization of test methods based on FDA, Ph. Eur., and WHO requirements. Also, there are several examples in the guidance; whereby, we would appreciate efforts to keep in line with ICH guidance.

Further, we acknowledge that therapeutic cancer vaccines are not considered in this guidance. However, in the absence of similarly detailed guidance for such types of products, this guidance could potentially influence the thinking of FDA review teams for vaccines manufactured using cell substrates that are outside its scope (e.g., ALVAC cancer vaccines).

On behalf of sanofi pasteur, we appreciate the opportunity to comment and thank you for your consideration of these responses. Should you wish to discuss any of our comments or concerns further, please address inquiries directly to Denise Rieker, Director, Regulatory Policy and Intelligence, by telephone at (570) 895-3465.

Sincerely,

A handwritten signature in black ink, appearing to read "D. L. Rieker". The signature is fluid and cursive, with the first letters of the first and last names being capitalized and prominent.

Denise L. Rieker  
Director, Regulatory Policy and Intelligence

DR/kh

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Section, Line, Paragraph	Current Text	Comment/Proposed Change
II.B.1 §2	“You should validate any methods used to inactivate or clear potential viral contaminants during production of your vaccine including the starting materials used to produce it, as the purity of your product could be affected by the purity of reagents and biological raw materials you use to produce the vaccine.”	“Methods used to inactivate or clear potential viral contaminants during production of the vaccine, including starting materials used to produce it, should be validated, as the purity of your product could be affected by the purity of reagents and biological raw materials you use to produce the vaccine.”
II.B.1 §2	“Certificates of Analysis (COA) for all reagents and biological raw materials used for vaccine production should be included in your submission.”	It is not clear if this request applies to all reagents or only to those of animal origin; thus, we suggest the following: “Certificates of Analysis (COA) for all biological reagents and biological raw materials used for vaccine production should be included in your submission.”
II.B.4 § 1& 2	“If you are using primary cell cultures to propagate your vaccine virus, complete testing of the primary culture might not be feasible prior to inoculation of virus...Use of control-cell cultures is important when your vaccine when your virus might interfere with the results of in-process testing of the product; for example, when the virus cannot easily be neutralize to permit testing for adventitious agents.”	Clarification is requested regarding application of this statement for continuous or diploid cell lines, as well as primary cell cultures.
II.B.4 § 2	“You should propagate control-cell cultures under conditions similar to production for a suitable period...(rest of paragraph).”	Clarification is also requested for cells in suspension, as observation of these cells can be technically difficult. Further, the relevance of the test may be questionable in this case, as handling control cells in such conditions may not be similar to those used in production.
III.A.2	“For example, neuronal cells might harbor latent viruses (e.g., herpesviruses) or express infectious prion proteins (PrP) and should be evaluated for these	We would appreciate suggestions from the Agency on how to accomplish that, as well as clarification on what other “additional considerations” might apply to tumor-

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	potential adventitious agents. Additional considerations might apply to cells that are tumor-derived.”	derived cells.
III.A.2	“You should also provide the medical history of the donor and the results of any screening and testing performed on the donor or on samples from the donor.”	It may be difficult to obtain information concerning the donor’s medical history and even ethically challenging in cases of cells of embryonic or fetal origin.
III.A.2 §3	“You should also provide the following: <ul style="list-style-type: none"> <li>- age, gender, and species of the donor;</li> <li>- donor’s medical history and the results of tests performed on the donor for the detection of adventitious agents.”</li> </ul>	It may be difficult to obtain information concerning the donor’s medical history and even ethically challenging in cases of cells of embryonic or fetal origin. Clarification is requested regarding what is to be done if part or all of this history is not available.
III.B.1 §5	“You should document in your biologics license application the location, identity and inventory of individual ampoules of cells.”	We agree that location and identity should be documented in the license application; however, we request clarification on the level of information required regarding inventory, as the amount of individual aliquots of cells constantly changes.
III.B.4 §1	“Diploid cell strains are established from primary cell cultures by expansion and cell banking. These types of cells have a finite life span and or not immortal like cell lines. Diploid cells usually retain a diploid or near diploid karyotype, a characteristic that also differs from cell lines, which are generally aneuploid or non-diploid.”	Embryonic stem cell-derived substrates retain diploid karyotypes and other properties of diploid cells with an infinite life span. Consideration of these is requested.  Further, clarification is requested regarding the definition of a cell line derived from embryonic stem cells without genetic, viral or chemical engineering, which has a normal, stable diploid karyotype and is capable of long-term proliferation in culture with no changes in growth kinetics. Such cells do not fit either the definition of diploid cells or the definition of a continuous cell line as given in the guidance. Some clarification of acceptable residual host cell DNA levels from such a cell substrate

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		is requested.
III.C §2	“Viral seeds should be stored in liquid nitrogen and in more than one location within a manufacturing facility or at a distant site for security reasons.”	We suggest the guidance be less specific with respect to storage requirements, i.e., “under appropriate conditions for the specific seed type and presentation”, as certain viral seeds may not be stored in liquid nitrogen.
III.C.1 §1	“You should extensively characterize your MVSs.”	We acknowledge the flexibility in the section entitled “Cell Banking Strategies and Methods” pertaining to the MCB and WCB and would appreciate the same consideration for this section. Thus, we suggest the following text, “You should extensively characterize your MVSs or WVSs.”
III.C.1 §1	“In addition, you should demonstrate the stability of genotype and phenotype for a number of passages beyond the level used in your production.”	Clarification is requested regarding acceptable criteria for mutation level and how such test results could be interpreted, as well as if this pertains to both attenuated live virus vaccines and inactivated vaccines?
III.C.1 §3	“Preferably, neutralizing antibodies should be monoclonal and prepared in a species other than the cells in which the MVS was prepared.”	“Preferably, neutralizing antibodies should be monoclonal and if polyclonal prepared in a species other than the cells in which the MVS was prepared.”
III.C.2	“You may subject working virus seeds (WVS) to less rigorous characterization than the MVSs from which they were derived.”	We acknowledge the flexibility in section entitled “Cell Banking Strategies and Methods” pertaining to the MCB and WCB and would appreciate the same consideration for this section. Thus we suggest the following text, “If you choose to extensively characterize your MVS, you may subject working virus seeds (WVS) to less rigorous characterization than the MVSs from which they were derived.”
III.C.3.e §2	“In addition to testing he viral or vaccine bulk...”	Typographical error: “he” should be “the”

<p>III.E.3 §1&amp;3</p>	<p>“In general, the stage at which adventitious agents are most likely to be found is the stage...(rest of paragraph)”                  “As discussed for the MVS (Section III.C.1), if the assay system used for in vitro or in vivo adventitious virus testing...(rest of paragraph.”</p>	<p>Adventitious agent testing, in vitro and in vivo, is usually not required for production lots of inactivated vaccines, as the MVS or WVS and MCB or WCB are controlled. Such testing is performed on production lots of live attenuated vaccines; thus, we suggest clarifying and specifying that in the guidance.</p>
<p>IV.A.1</p>	<p>“In the development of viral vaccines, in vivo adventitious agent testing includes inoculation...(rest of paragraph)”</p>	<p>Some differences are observed compared to WHO requirements (e.g., volume inoculated, test article inoculated for cell bank, number of animals to be injected). Harmonization would be helpful.</p>
<p>IV.A. §2</p>	<p>“For each of the suggested adventitious agent tests, alternatives such as those recommended by the World Health Organization (WHO) or the European Pharmacopoeia (EP) might be considered if justified with data showing sensitivity comparable to the recommended test.”</p>	<p>Comparability data are usually not available for FDA, Ph. Eur., and WHO-required testing. Rationale for test differences is often historic. We request that for newly introduced tests (e.g., Mycobacteria) there be harmonization up front, based on an existing regulation.</p>
<p>IV.A.1.c §4</p>	<p>“In vitro methods, such as culture and PCR, are also acceptable for identifying <i>Mycobacterium tuberculosis</i> when validated.”</p>	<p>Clarification is requested, as we believe this test to be suitable for detection of possible contamination but not for identification.</p>
<p>IV.A.1.e</p>	<p>“A sample volume, equivalent to at least 100 doses, or 10 mL, whichever represents a greater volume, should be used in testing. At least 10 embryonated eggs, 10 to 11 days old, should be inoculated by the allantoic route using 0.5 mL per egg.”</p>	<p>In some cases the sample volume may be limited. Also, the use of 0.5 mL to inoculate may result in trauma to the embryo, subsequently invalidating the test. We suggest allowing for alternative sample volumes and egg inoculums, provided that required sensitivity can be demonstrated.</p>
<p>IV.A.2.a Methods §1</p>	<p>“An appropriate volume should be inoculated onto monolayer cultures of at least 3 cell types...”</p>	<p>For human (e.g. MRC-5) and simian cells (e.g. Vero) based vaccines, the usefulness of a third cell system is</p>

		not clear since cell banks or viral seeds will be tested on both MRC-5 and Vero cells.
IV.A.2.a Methods	Entire section	<p>Clarification is requested regarding the usefulness of a subculture, as it was not demonstrated that the subculture increases the sensitivity of the assay with model viruses in many cases. The guidance indicates that the subculture might help in reading CPE in case of toxic effects of the initial specimen onto the cells. However, these toxic effects are not frequent and are product-dependent.</p> <p>It is likely more reasonable to implement a PCR test for simian CMV or human CMV, as opposed to extending the duration of the test to 4 weeks.</p> <p>Clarification is requested with respect to different recommendations for cell substrates and viral seeds, particularly regarding hemagglutination versus hemadsorption practices. One suggestion is to perform these tests using a pool of red blood cells.</p>
IV.A.2.b	“TEM can detect viral particles in a cell substrate, including those from endogenous retroviruses. Under some circumstances, it might be appropriate to pre-treat cells with chemical or inducing agents to activate production of endogenous or latent viruses.”	Clarification is requested regarding these “circumstances”. Should an agent such as IUdR be used to activate latent retroviruses for all cell substrates?
IV.A.2.c §4	“CBER recognizes that some products and reagents have RT activity that does not to represent adventitious infectious retroviruses... (rest of paragraph)”	We would appreciate confirmation that this test is required on cell substrates. For avian cell substrates, it is recommended to test the cell substrate with an appropriate test to assure the absence of major avian retroviruses. This test is usually feasible on cell

		<p>substrates but very difficult to perform on viral seeds or harvest due to neutralization issues.</p> <p>Typographical error: The word “to” is not necessary.</p>
IV.A.2.d	<p>“Under some circumstances, for example when tumorigenic cell substrates are proposed for use, it might be appropriate for you to pre-treat cells with chemical agents known to induce reactivation or replication of endogenous or latent viruses?”</p>	<p>Clarification is requested regarding these “circumstances”. Should an agent such as IUdR be used to activate latent retroviruses for tumorigenic cell substrates before performing an infectivity test?</p>
IV.A.3.a, c	<p>Entire section</p>	<p>Please consider harmonization with Ph. Eur. Specifically, the Mycobacteria Testing section shows differences in sample volume (2.0 mL vs. 2.7 mL), test duration (6 weeks vs. 56 days), and media.</p>
IV.B.1 §4	<p>“You should use an animal model that is known to be susceptible to tumor formation by tumorigenic cells. Because immunocompromised adult and newborn rodents are relatively sensitive for revealing a tumorigenic phenotype, you should consider these animal models. Thus, the most commonly used animals for tumorigenicity testing are nude (nu/nu) mice because they are T-cell deficient. Newborn nude mice appear to be more susceptible to tumor formation than adult nude mice (Ref. 22), suggesting that newborn nude mice might be the best choice to use when identification of a weakly tumorigenic phenotype is important. You might choose to use another animal model if it has been shown to have comparable sensitivity to the nude mice model</p>	<p>In the FDA PTC (1993), the WHO TRS 878 (1998) and the Ph. Eur. 5.2.3, as well as the FDA letter to manufacturers (2001), different animal models are proposed to test the tumorigenicity of cells, including immunosuppressed rats. The test in immunosuppressed rats was extensively used to describe the tumorigenicity of Vero cells. We suggest its continuation without the need for demonstrating that its sensitivity is comparable to the test in nude mice, as this model was shown to be very sensitive.</p>
IV.C.1	<p>“You should test your final vaccine product for the</p>	<p>Please verify that testing of final vaccine product for</p>

	<p>presence of residual cells. Processes, such as filtration, should be implemented and validated to ensure that intact cells are not present in the final product. Validation that residual cell removal processes are robust is important for immortalized cells. Determining the extent to which intact cells are cleared by these processes is an important part of this validation.”</p>	<p>presence of residual cells is not a routine requirement if the process is validated for such.</p>
IV.C.2 §1	<p>“Residual DNA also might be capable of transmitting viral infections if retroviral proviruses, integrated copies of DNA viruses, or extrachromosomal genomes are present.”</p>	<p>Clarification is requested that only infectious (exogenous) retroviral proviruses are a concern. Avian cells contain large numbers of defective endogenous proviruses that are not known to be hazardous to humans. While it is feasible to screen flocks for exogenous infectious retroviruses, it would not be practical to attempt to avoid endogenous defective provirus.</p>
IV.C.2 §3	<p>“We might require limitation of the amount of residual DNA, depending on the potential risks associated with that DNA, for human diploid or primary cell types for which there is less experience.”</p>	<p>We acknowledge that inclusion of this potential requirement in the guidance is likely to minimize the infectivity risk, as opposed to the oncogenicity risk, associated with DNA. However, for primary chick embryo fibroblasts, for example, the cells are usually obtained from SPF eggs, and control cells are tested for adventitious agents including retroviruses, thus reducing the risk of infectivity from the host cell DNA. We would appreciate clarification as to whether or not this potential requirement would be relevant in this case.</p>
IV.C.2 §3	<p>“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 (ref. 28).”</p>	<p>We reference the November 16, 2005 VRBPAC meeting; whereby, CBER stated (cf transcript p. 43): “So for Vero cell produced vaccines that are intended to be given parenterally, we would like to see fewer than 10 nanograms per dose. The same is true for the vaccines</p>

		that are produced in the 293 or PER.C6 cells”. We suggest that the DNA content limit of 10 ng/dose for low tumorigenic cells (e.g., HEK 293 and PER.C6) be mentioned in this guidance.
IV.C.3	“The requirements of a GST are described in 21 CFR 610.11. For vaccines, an exemption to the GST may be requested, as specified in 21 CFR 610.11(g)(2).”	Please consider harmonization with Ph. Eur., specifically with respect to sample volume. Test performance could be increased with an injection volume in the guinea pig of one human dose, as opposed to a fixed volume of 5 mL.
VII. Reference List	References 11 and 14	Update to 5 <sup>th</sup> edition and replace Section 2.6.6 with 2.6.16 .
Appendix 1	Table I – general	<p>We suggest that inactivated viral vaccines be distinguished from live viral vaccines. Also, distinguish between vaccines produced in primary cells from those produced in control cell banks.</p> <p>Mycoplasma/spiroplasma, residual cellular protein and residual cellular DNA testing are usually performed at the vaccine bulk stage.</p>