United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Washington, DC 20250

VETERINARY SERVICES MEMORANDUM NO. 800.117

TO: Veterinary Services Leadership Team
Directors, Center for Veterinary Biologics
Biologics Licensees, Permittees, and Applicants

FROM: John R. Clifford *[/s/ John R. Clifford, 8-12-2013]*
Deputy Administrator

SUBJECT: Guidance for Inactivation Studies

I. PURPOSE

This memorandum provides guidance for designing studies supporting the confirmation of the inactivation process for inactivated bacterial products and killed virus vaccines.

II. POLICY

Title 9, *Code of Federal Regulations* (9 CFR), section 113.200(a), states that for Killed Virus Vaccines "suitable tests to assure complete inactivation shall be written into the filed Outline of Production." Further, 9 CFR 113.100(a)(1) states that "…an inactivated bacterial product shall be tested for viable bacteria and fungi as provided in [9 CFR]113.26." The Center for Veterinary Biologics (CVB) interprets these regulations to mean that the process of inactivation must be consistent and supported by suitable studies. The appendix to this Memorandum describes a suitable approach for accomplishing this requirement.

III. IMPLEMENTATION/APPLICABILITY

Implementation is as of the date of this Memorandum. Guidance for Inactivation Studies is appended to this Memorandum. This guidance applies to products in the prelicense stage, except for those prelicense products whose inactivation procedures have been deemed satisfactory before the implementation date. The guidance also applies to licensed products whose manufacturing process or inactivation procedure is undergoing significant changes. This guidance does not apply to currently licensed products whose inactivation procedures remain unchanged. The guidance does not create new requirements; alternative approaches may be proposed.

Attachment: Appendix

Appendix

Guidance for Inactivation Studies

1. Objective

These guidelines provide guidance for developing suitable studies and testing procedures to demonstrate that the inactivation procedure described in the manufacturer's Outline of Production will consistently inactivate the infectious agent.

2. Background

2.1. *Inactivation kinetics.* Inactivation kinetics of microorganisms can only be directly observed up to the limit of detection (LOD) of the titration or assay method. Since practical inactivation endpoints are below the LOD, it is difficult to estimate the time until the targeted endpoint is achieved. Extrapolation of the inactivation curve to the endpoint is unsatisfactory for several reasons. The observed inactivation curves may be difficult to fit accurately due to their potentially multiphasic nature, and there are technical uncertainties in extrapolation itself. Most importantly, it is not unusual for the inactivation kinetics to change beyond the LOD. For practical reasons we describe the inactivation kinetics of a biological agent as composed of two stages: the first stage, when kinetics may be directly observed; and the second stage, when the rate of inactivation is not directly observed.

Goal. Since in practice it is difficult to directly determine the rate of inactivation in the second stage, one has to adopt a target intended to minimize the risk of infecting the vaccinated population and exposing non-vaccinates to residual live agent. A common target is less than one infectious unit per million doses ($1x10^{-6}$ infectious units/dose), and that is the working target underlying these guidelines. Note, however, that it is not straightforward to relate the population risk to the target because of various uncertainties of several factors that vary widely between antigens, products, and lots, such as the number of viable organisms in an infectious unit and the volume of bulk antigen in the final vaccine dose. For that reason, the confirmation of inactivation testing performed on each vaccine lot is also an important part of the ongoing monitoring of the inactivation process.

3. Definitions

3.1. *Inactivation Curve.* The inactivation curve describes the relationship between the number of live particles and time of the inactivation process. The observed part (first stage) of the curve is the part obtained by conducting titrations at regular intervals after the start of inactivation. The unobserved part (second stage) of the curve is the part below the titration's LOD.

3.2. *Endpoint*. The end of a sequence of five consecutive negative second-stage tests taken at least 1 hour apart.

3.3. *Time to endpoint (TE).* In a trial, the duration of exposure to the inactivation process to reach the endpoint.

3.4. *Required time of inactivation (RTI).* The required time of exposure to the inactivation process. The RTI is the maximum observed TE of at least three trials.

3.5. *First stage assay.* This is the titration or colony count testing procedure used to estimate the observed part of the inactivation curve and determine when the LOD is reached.

3.6. *Second stage assay.* This is a yes-or-no test that detects the presence or absence of the live agent at very low concentrations in a specified volume of material. It incorporates one or more concentration and amplification steps. In each trial, this binary assay is used during the second stage of the inactivation process to determine the TE.

4. **Study Design**

Inactivation studies supporting the consistency of the inactivation procedures proposed in the Outline of Production must be conducted under conditions representative of actual production conditions. The guidance below represents practices the CVB considers valuable for evaluating the consistency and estimating the RTI, but it does not cover all possible scenarios. The CVB recommends that firms submit a draft protocol and preliminary data, if available, before initiating final studies.

4.1. *Number of trials.* Three trials.

4.2. *Batch size.* Two batches ≥10 percent; one ≥ 33 percent of the maximum production batch size. Data from smaller-scale batches used to identify and optimize the conditions for inactivation may be submitted as supporting data.

4.3. *First-stage testing.* The goal of first-stage testing is to characterize the observable part of the inactivation curve and determine the time when it reaches the LOD. Sampling should begin just before the start of the inactivation process and continue at regular intervals until the LOD is reached. Nine sample points are recommended, collected in duplicate. Include in the report the sample volume, and express the titer or count as per mL of the original culture, correcting for dilution and concentration steps. To maximize sensitivity a concentration step is recommended. Some

inactivation processes are very rapid, so it may not be feasible to have nine sampling points.

4.4. *Second-stage testing.* The goal of second-stage testing is to determine the TE of individual trials, and to use them to estimate RTI. Planning the initiation and timing of second-stage testing is based on preliminary studies, which assist in determining the observable and unobservable portions of the inactivation curve. Second-stage testing should begin approximately at the time when the first-stage testing reaches the LOD of the method. The TE is reached when five consecutive negative test results are obtained. These are performed in duplicate at least 1 hour apart using a minimum sample volume of 100 mL for most production procedures.

4.5. *Inactivation conditions.* Describe the inactivation conditions in detail. Some or all of the following conditions may be important for defining the inactivation process, depending on the inactivating agent and the microorganism. Additional parameters may be necessary to fully describe the process.

4.5.1. *Stage of culture.* Conduct the inactivation studies on cultures harvested at the concentration (titer or count) and at the stage of growth specified in the Outline of Production. For purposes of validating the inactivation process, the concentration should be near the highest expected concentration described in the Outline of Production.

4.5.2. *pH.* Conduct the inactivation studies at the pH specified in the Outline of Production. This is usually ± 0.2 pH unit.

4.5.3. *Sample treatment and neutralization steps.* Describe sample processing, including the concentration and neutralization steps. State how neutralization of the inactivation agent is determined.

4.5.4. *Protein content.* The goal is to have consistent conditions and minimize interference by extraneous protein. If the cells or virus particles are washed before treatment, indicate the washing steps. If a protein assay is used to establish the protein content, specify the range.

4.5.5. *Temperature.* Specify the temperature at which the inactivation procedure is conducted (± 2°C).

4.5.6. *Osmolality.* Specify the osmolality or describe the composition of the medium used during the inactivation process (e.g., twice-washed culture fluids were suspended in 0.15 M NaCl 0.05 M Na/K phosphate buffer pH=7.15). The goal is to have consistent conditions and minimize interference by variations in the osmolality of the medium.

4.5.7. *Conditions of exposure to the inactivating agent.* The length of exposure to the inactivating agent should be specified in terms of concentration of the inactivating agent and time (hours and minutes) for the initial and any repeat exposures. Also, indicate adjustments of the medium pH, neutralization steps, and washing steps.

4.5.8. *Facilities description.* Describe the physical environment and equipment used for the inactivation process, so that it is consistent from batch to batch. This may include a description of the room and precautions taken to isolate it from production and finished product. Describe the incubator, inactivation vessels, (size, geometry), monitoring systems, mixing equipment, control of conditions, and if the fluids are transferred to a second or third vessel.

5. **Summary**

Preliminary test runs of the inactivation process using smaller batch sizes are essential to estimate conditions before confirming in larger batches. Confirm the consistency of the inactivation process in a minimum of three batches prepared according to the Outline of Production. Two batches must be ≥ 10 percent and the third ≥ 33 percent of the maximum batch size. Test for residual viability with a quantitative or quantal method (first stage) and follow with a yes-or-no test method (second stage). The first test method is used to show the initial kinetics of inactivation is consistent and to determine when testing with the second method should begin. The second test method is used to determine the endpoint and should incorporate concentration and amplification steps and replicate sampling of a minimum of 100 mL of culture. Sampling and testing continues at a minimum of 1-hour intervals until five negative tests in a row are obtained. The TE is the time of the fifth negative test and the RTI is the maximum TE for the three batches. The 1-hour interval is a minimum; intervals may be wider.

6. **Report**

Submit a report that describes the inactivation process, the material and methods used in the study, and the limit of detection of the first- and second-stage testing. Summarize how the limit of detection was determined, the results of each trial, and the analysis of the results. The report should also include a detailed procedure for the confirmation of inactivation test method performed during manufacture of the product. This test should be very similar to the second-stage assay method in terms of sample size, concentration, and amplification steps. Submit all data in a useable format.

7. **Monitoring**

The confirmation of inactivation testing of production serials will provide important additional verification of the consistency of the inactivation procedure.