Animal and Plant Health Inspection Service

Veterinary Services

1400 Independence Ave, SW

Washington, DC 20250

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VETERINARY SERVICES MEMORANDUM NO. 800.118

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

FROM: John R. Clifford */s/ Jack A. Shere, for*
Deputy Administrator

SUBJECT: Live Master References

I. PURPOSE

This memorandum provides guidance for using live cultures of microorganisms as Master References in potency tests of inactivated products and describes recommendations for monitoring their stability.

II. BACKGROUND

Veterinary Services (VS) Memorandum No. 800.211, appendix, section 4.2 allows continuous use of Master References of products licensed after January 1, 2011, if the Master Reference remains stable. VS Memorandum No. 800.112, appendix III, section 2.4 expands on this concept by identifying the type of testing necessary to demonstrate the Master Reference remains stable. It specifies that validated quantitative and qualitative test methods must be used as part of the stability-monitoring program.

Ideally, potency tests of inactivated vaccines would provide a quantitative measure of the protective antigen. This is not always possible because of the complexity of vaccine formulations and limitations of the analytical methods. Instead, relative potency assays may be developed that compare a production lot of the vaccine to a reference lot of the vaccine. It is then necessary to monitor the stability of the reference. To do so in the absence of direct antigen quantitation, a preparation of the live microorganism prepared according to the Outline of Production may be used as a Master Reference if the live preparation performs the same in the validated potency assay as the inactivated antigen. The viable count or titer of this Live Master Reference would serve as a measure of its stability.

Title 9, *Code of Federal Regulations* (9 CFR), part 101.5(o) states that "The preparation of a Master Reference as described in a filed Outline of Production may be: (3) 'A non-adjuvanted harvested culture of microorganisms.'" The Center for Veterinary Biologics (CVB) interprets this statement to include live

cultures produced from the approved Master Seed according to the filed Outline of Production which may be used as Master References. The CVB refers to these preparations as Live Master References (LMR).

III. POLICY

The viability of the Live Master Reference will serve as a measure of antigen stability when it is qualified and monitored as described in the appendix to this memorandum.

IV. IMPLEMENTATION/APPLICABILITY

This policy is effective the date of publication of this memorandum.

Appendix

Appendix

Guidelines for Live Master References

1.1. Definitions

1.1.1. *Master Reference* (MR). Defined in 9 CFR 101.5 (o).

1.1.2. *Working Reference* (WR). Defined in 9 CFR 101.5 (p).

1.1.3. *Qualifying Serial* (QS). Defined in 9 CFR 101.5 (q).

1.1.4. *Live Master Reference* (LMR). A live culture of microorganisms used as a Master Reference.

1.1.5. *Independent Method*. Test methods that evaluate the analyte using an analytical principle that differs from the potency test.

1.1.6. *Semi-Quantitative Assay*. An assay method that expresses the results in an approximate way instead of in absolute terms. (For example, in a dot blot the signal of the reference is positive at a 1/50, 1/100, and 1/200 dilutions but disappears at a dilution of 1/400. The reference point is the presence of a signal at a 1/200 dilution and disappearance of signal at a 1/400 dilution under a standard set of conditions.)

1.2. Rationale

1.2.1. The viable count of the LMR is a measure of its stability. It is not a measure of potency, because the relationship between the number of viable organisms and amount of antigen is not a constant.

1.2.2. The relative potency of the LMR is established by qualification procedures similar to a non-viable MR.

1.2.3. In addition to viability testing, the stability of the LMR must be supported with evidence from independent qualitative and semi-quantitative validated assays, and periodic comparison of the LMR to contemporary serials in the validated potency test method.

1.3. LMR Qualification

1.3.1. Show the dose-response curves of the LMR in the validated potency assay are parallel to the curves of at least two representative serials or serial prototypes from each product code affected as per VS Memorandum 800.112, appendix III, section 2.2.5.

1.3.2. Show the LMR is related to host animal immunogenicity by

1.3.2.1. Showing the dose response curves of the LMR are parallel to a qualified, unexpired, MR in a validated potency test method. The LMR relative potency must be ≥ 1.0 at the use dilution when compared to the MR.

or

1.3.2.2. Conducting a host animal immunogenicity trial using a Qualifying Serial.

1.4. LMR Stability Monitoring

1.4.1. *Frequency.* Monitor the LMR for stability at 0, 3, 6, 9, and 12 months and then at 6-month intervals after qualification. Use the viability assay and qualitative/semi-quantitative assays. Also, track the LMR performance in the vaccine potency assay.

1.4.2. *Viability Assay.*

1.4.2.1. The viability assay measures the viable count or titer of the LMR. It must be validated following the principles in VS Memorandum No. 800.112.

1.4.2.2. The viability assay must be able to detect a change of 20 percent in viability.

1.4.2.3. For viability monitoring, the CVB has developed a template incorporating a statistical quality control method. (The method is a type of CUSUM, a trend monitoring procedure based on cumulative sums.) Alerts initially lead to a shorter interval for monitoring viability, and three consecutive alerts at the shorter intervals indicate a change in viability. The template and instructions for use are available on the CVB website.

1.4.2.4. Each operator conducting viability testing must first test a sufficient number of vials of the LMR to establish their performance parameters. This information is incorporated in the template.

1.4.3. *Independent Qualitative and Semi-quantitative Methods.* Use at least one validated qualitative and one validated semi-quantitative assay to evaluate qualitative/semi-quantitative parameters of the LMR that is relevant to the potency of the reference (VS Memorandum No. 800.112, appendix III, section 2.4) and independent of the potency test and viability assay methods. Establish acceptance criteria when validating the assay.

1.4.4. *LMR Stability in the Potency Assay.* The assessment should follow the validated test method with the exception that the dilution series should demonstrate the entire dose response curve from saturation through extinction for the LMR, two recently manufactured serials, and the working reference in two valid assay runs.

1.5. LMR Dating

1.5.1. The LMR will be considered stable and therefore remain qualified if it successfully completes the stability testing regimen. If the LMR changes in viability, is non-parallel to serials in the potency assay or has evidence of degradation as determined in the independent assessment assays, it will not be satisfactory for serial release and cannot be used to qualify a new Working Reference. This information must be reported immediately to the CVB, as

required by 9 CFR 116.5(b). A new LMR must be qualified in the target species by methods acceptable to the Animal and Plant Health Inspection Service.

1.6. Submissions for LMR Approval

1.6.1. For previously validated and approved relative potency assays, submit a supplemental report demonstrating the dose response curve of the LMR is parallel to the unexpired MR and two representative serials. The report should include the entire dose response curve from saturation through extinction for each sample tested.

1.6.2. For new potency assays, provide a validation report for the potency assay comparing the LMR, MR, or QS and two representative serials or prototypes of each related product. The QS must demonstrate satisfactory efficacy in order for the LMR to be accepted as the Master Reference.

1.6.3. For all submissions, provide validation reports for the viability assay and independent qualitative and semi-quantitative test methods.

1.6.4. Submit a report summarizing the stability monitoring of the LMR at 12 months and at 12-month intervals thereafter.

1.7. Use of Agents to Enhance Stability of the LMR

1.7.1. Chemical additives, cryo-preservatives, or storage conditions designed to enhance stability must have little or no effect on the approved potency test method.

1.7.2. The LMR may be stored as a concentrate, use-dilution, or at some intermediate concentration.

1.7.3. The summary information sheet for the approved LMR must specify the name, source, and concentration of each preservative, the storage conditions, container type, and composition.