

VETERINARY SERVICES MEMORANDUM DOC. NO. 351

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

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SUBJECT: Guidelines for Live Master References

I. PURPOSE

The purpose of this document is to provide guidance to firms describing the establishment of live cultures of microorganisms as Master References for *in vitro* assays used for serial release of inactivated products. This guidance document represents the agency's policy regarding issues related to Master References and reference stability.

II. SCOPE

The application of live culture derived from the approved Master Seed and prepared according to the filed Outline of Production as Master References is exclusively for inactivated products. Titration of the viable organisms present in the samples prepared for this purpose is exclusively for assessing stability of the stored material and is not intended to represent a measure of product potency.

III. BACKGROUND

Title 9, Code of Federal Regulations (9CFR), Part 101.5(o)(3) states that “a nonadjuvanted harvested culture of microorganisms” may serve as a Master Reference. The Master Reference may be used in *in vitro* potency assays directly for serial release or may be used to establish the potency of a Working Reference. Master References usually consist of material produced according to the filed Outline of Production through the stage where the microorganism has been inactivated by physical or chemical means. The Center for Veterinary Biologics interprets this statement to include live cultures produced from the approved Master Seed according to the filed Outline of Production. These live cultures stored under stringent conditions may be used as Master References in *in vitro* potency assays for serial release, or to define the potency of Qualifying Serials or Working References.

IV. GLOSSARY

Master Reference. A Master Reference is a reference whose potency is correlated, directly or indirectly, to host animal immunogenicity. The Master Reference may be used as the Working Reference in *in vitro* tests for relative potency. The Master Reference may also be used to establish the relative potency of a serial of product used in requalification studies and to establish the relative potency of Working References. The preparation of a Master Reference as described in a filed Outline of Production may be:

- A completed serial of vaccine or bacterin prepared in accordance with a filed Outline of Production; (9CFR 101.5 (o)(1))
- A purified preparation of a protective immunogen or antigen; (9CFR 101.5 (o)(2))
- A nonadjuvanted harvested culture of microorganisms. (9CFR 101.5 (o)(3))

Working Reference. A Working Reference is the reference preparation that is used in the *in vitro* test for the release of serials of product. Working References may be:

- Master References or
- Serials of product that have been prepared and qualified, in a manner acceptable to Animal and Plant Health Inspection Service for use as reference preparations.

Qualifying Serial. A serial of biological product used to test for immunogenicity when the Master or Working Reference is a purified antigen or nonadjuvanted harvest material. (9CFR 101.5 (q))

Live Master Reference (LMR). A preparation of the approved Master Seed produced according to the filed Outline of Production at the highest permissible passage, harvested prior to the inactivation step, and qualified as described below.

Independent Method. Test methods that evaluate physicochemical properties of the analyte that differ from the potency test or assess the same properties in a different manner.

V. GUIDELINES FOR IMPLEMENTATION OF A LMR

Approval of the LMR for serial release requires it be qualified as a Master Reference and a program developed for monitoring its stability. (See flow chart in Appendix.)

Qualification

There are four parts to qualifying a LMR for use as a Master Reference.

- Demonstrating the LMR is correlated to host animal immunogenicity by using a Qualifying Serial and conducting a host animal immunogenicity trial or showing the dose response curve of the LMR is equivalent to a qualified, unexpired Master Reference in a validated potency test method.
- Showing the LMR performs the same as at least two representative serials or serial prototypes for each product code affected by the validated potency test method with respect to parallelism of the dose response curves.

- Quantifying the number of viable organisms present in the LMR at the time of the host animal immunogenicity or the comparison to the qualified Master Reference using a validated viability assay method.
- Developing and conducting an independent assessment of qualitative and semi-quantitative characteristics of the antigen in the LMR using validated test methods.

Once the LMR is qualified, it may be used for serial release, qualify a Working Reference or qualify a Qualifying Serial.

Viability Assay

- The viability assay must be a validated test.
- Use a dilution factor no greater than 5/4.
- Establish the time zero value of the number of viable organisms in the LMR at the time of the host animal immunogenicity study or when the comparison with the qualified Master Reference is conducted.

Independent Qualitative and Semi-quantitative Methods

At least one validated qualitative and one validated semi-quantitative assay that are independent of the potency test and viability assay method must be used to periodically evaluate the stability antigen in the LMR.

These methods must be approved by APHIS at the time of the qualification.

VI. STABILITY MONITORING

LMR Viability

- Assess the viability of the LMR using the approved viability assay at 0, 1, 3, 6, and 12 months and then at 4-month intervals following qualification of the LMR.
- At each time interval, estimate the number of viable units by taking the geometric mean titer (GMT) of enough replicate tests so that the standard error of $\log_d(\text{GMT})$ is no greater than 0.2, (where d is the dilution factor).
- The LMR is satisfactory for continued use if
$$|\log_{10}(\text{GMT}_{\text{current}}) - \log_{10}(\text{GMT}_0)| < 0.05$$

LMR Stability in the Potency Assay

The LMR must be evaluated at 0, 1, 3, 6, and 12 months and then at 12-month intervals following qualification of the LMR.

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.

LMR's qualified by comparison to an unexpired Master Reference should be compared to the Master Reference while it remains in dating. In addition, include two recently manufactured production serials.

LMR's qualified as *de novo* Master References should be compared to two recently manufactured production serials.

The LMR must have a dose response curve parallel to the Master Reference and two production serials in two valid assay runs.

Independent Qualitative and Semi-quantitative Methods

The LMR must be evaluated at 0, 1, 3, 6, and 12 months, then at 12-month intervals following qualification of the LMR. Possible methods for assessing qualitative and semi-quantitative parameters include electrophoretic analysis, column chromatography, other immunoassays, or other methods acceptable to APHIS. Method selection must minimally address a qualitative and semi-quantitative feature of the antigen in the LMR.

VII. DATING OF THE LMR

The LMR will be considered stable and, therefore, qualified if it successfully completes the periodic Viability Assay, Potency Assay, and Independent Qualitative and Semi-quantitative assessments. If the LMR changes in viability, parallelism 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 should be reported to CVB as required by 9CFR 116.5(b). A new LMR must be qualified in the target species by methods acceptable to APHIS.

VIII. SUBMISSIONS

For previously validated and approved potency assays, submit a supplemental report demonstrating the dose response curve of the LMR parallel to the Master Reference, Qualifying Serial (if not expired), and two representative serials. The report should include the entire dose response curve from saturation through extinction for each sample tested.

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

For all submissions, provide validation reports for the Viability Assay and Independent Qualitative and Semi-quantitative test methods.

Reports summarizing the stability of the LMR must be submitted at 12 months, 5 years, and at 5-year intervals thereafter.

A flow chart summarizing the steps necessary for qualifying a LMR is in the Appendix.

IX. USE OF AGENTS TO ENHANCE STABILITY OF THE LMR

Chemical additives, cryo-preserved, or storage conditions designed to enhance stability must have little or no effect on the approved potency test method. The LMR may be stored as a concentrate, use-dilution, or at some intermediate concentration.

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, the volume of LMR, date of preparation, titer, assay method, assay precision, date of approval, temperature monitoring system, use dilution, monitoring test methods, and tolerances.

APPENDIX
Scheme for Developing and Maintaining an LMR

