May 26, 2004

VETERINARY SERVICES MEMORANDUM NO. 800.109

Subject: Master Seed and Master Cell Stock Testing Report Submission

To: Biologics Licensees, Permittees, and Applicants
Directors, Center for Veterinary Biologics

I. PURPOSE

This document provides guidance for submitting Master Seed (MS), Master Cell Stock (MCS) and Master Sequence (MSQ) test reports to the Center for Veterinary Biologics (CVB) for review and filing.

II. DEFINITIONS

A. Applicable 9 CFR definitions:

1. *Master Seed (MS),* 9 CFR 101.7 (a). An organism at a specific passage level which has been selected and permanently stored by the producer from which all other seed passages are derived within permitted levels.

2. *Master Cell Stock (MCS),* 9 CFR 101.6 (d). The supply of cells of a specific passage level from which cells for the production of biologics originate.

B. Definition of non-codified term:

Master Sequence (MSQ). The target sequence used to produce a synthetic reagent used in the production of a biologic.

III. BACKGROUND

All microorganisms and cell lines used in the manufacture of veterinary biological products must be derived from approved MS and MCS. Synthetic reagents must be produced from an approved MSQ. To obtain CVB approval of MS, MCS and MSQ, the licensee or permittee must submit a report of the testing performed according to Title 9, Code of Federal Regulations (9 CFR) or as specified in the filed Outline of Production. These results must be reviewed and filed by the CVB before the licensee or permittee is authorized to submit MS, MCS, or MSQ for confirmatory testing.

IV. GUIDELINES FOR TESTING

A. Master Seed and Master Cell Stock

1. *General requirements* – Refer to 9 CFR 113.64, 113.100, 113.200, and 113.300.

2. *Purity testing* – Refer to 9 CFR 113.26 and 113.27.

3. *Extraneous agents testing* – Refer to 9 CFR 113.46, 113.47, 113.52, 113.55 and product-specific Standard Requirements under 9 CFR part 113.

B. Master Sequence Characterization

Procedures for characterizing Master Sequences should be described in the filed Outline of Production or filed Special Outline.

V. GUIDELINES FOR SUBMISSION

The following sections describe the items that must be submitted to the Center for Veterinary Biologics – Policy, Evaluation and Licensing (CVB-PEL) for review of any MS, MCS or MSQ. The submission should include a summary of all the tests performed; a Veterinary Biologics Production and Test Report (APHIS Form 2008) may be used for this purpose. Additionally, a supporting report, detailing the materials, methods, results, and conclusions for each test, is required. Two copies of the report should be submitted.

A. Master Seeds:

1. *Seed Origin and Passage History*: Provide details of where the seed was obtained and passage history. The passage history must include how the organism was initially isolated and a summary of the passages since isolation, including the type of medium, cell culture, and/or animal used.

2. *Identity*: Each Seed must be identified to the taxonomic level specified by the 9 CFR or a level acceptable to APHIS. If the Seed has a particular strain or type designation, additional testing to confirm the strain/type must be performed. If the Seed expresses a particular antigen which is critical for immunogenicity (or as a marker) and which is not possessed by all isolates (e.g., K99 pilus on *Escherichia coli*), provide test results to confirm the expressions of this antigen.

3. *Purity*: Purity is tested according to the 9 CFR 113.27 for the detection of bacterial and fungal contamination. Additionally, viruses and other obligate intracellular organisms must be tested according to the 9 CFR 113.28 for the detection of mycoplasma contamination.

4. *Extraneous agents*: All viruses and obligate intracellular organisms must be tested for extraneous agents according to 9 CFR 113.55. Each virus of avian origin must also be tested for contamination with *Salmonella*, lymphoid leucosis virus, and hemagglutinating viruses according to 9 CFR 113.30, 113.31 and 113.34, respectively.

5. *Summary Information Formats* (SIF): For all Master Seeds produced by recombinant technology and the Master Seeds to be used in the production of live biological products, additional safety and identity data must be submitted in the form of a SIF; see Veterinary Services Memoranda 800.50 and 800.205. A complete SIF is required prior to licensure. However, an initial version of the SIF must be submitted concurrently with the Master Seed report. The SIF must contain adequate data for the CVB to establish proper bio-containment requirements and to conduct confirmatory testing. Submit two copies of the SIF. The SIFs for different categories of recombinant veterinary biologics, and conventionally derived, modified live veterinary biologics, are available at www.aphis.usda.gov/vs/cvb/lpd/sifs.htm. Recombinant organisms intended to express a foreign antigen(s) must be tested for that expression.

6. *Addenda*: Master Seeds used in modified live products may have codified test and retest requirements for immunogenicity, as defined in the applicable product-specific section of 9 CFR 113. When an immunogenicity study is completed for such a seed, an addendum to the original Master Seed report must be submitted. The addendum must include a summary of the results, including the Product Code in which the seed was tested, and the date of the study. If there are retest requirements for the Seed (repeat immunogenicity), then the addendum also must specify the year in which the repeat study should be performed. After the retest study is completed, a second addendum summarizing the study must be submitted. A brief summary of back passage study results must also be included as an addendum to the Master Seed report.

B. Master Cell Stock:

The general testing guidelines for cell lines are in 9 CFR 113.52. Cells must be completely characterized at their baseline passage level (X). The maximum passage level permitted for use in production (X+n) must be tested for karyology. The submitted report must include all of the following:

1. *Propagation*: Include detailed information regarding the medium, any special growth enhancer, or other characteristics specific to the MCS.

2. *Passage*: Include the origin and passage history of the cell line. Specify the baseline passage level of the Master Cell Stock (X).

3. *Purity*: The guidelines specified in 9 CFR 113.26 must be followed for bacterial and fungal contamination, and 9 CFR 113.28 for mycoplasma contamination.

4. *Identity and species specificity*: Detail the test method used to identify the cell line.

5. *Extraneous agent testing*: Testing must be done according to 9 CFR 113.46 and 113.47. List all the cell types (embryonic, neonatal, etc.,) used in testing and all potential contaminating viruses for which testing was conducted.

6. *Karyology*: Perform a chromosomal examination of at least fifty cells undergoing mitosis. Any chromosomal marker detected in the baseline passage must also be found in the highest passage cells. Determine the modal number for X and X+n passages. The modal number of chromosomes must not differ more than 15% in the highest passaged cells compared to the baseline passage.

7. *Tumorogenicity/Oncogenicity*: If there is any indication that the cell line may induce malignancy in the species intended for use, a tumorogenicity/oncogenicity test must be conducted by a method acceptable to APHIS.

C. Master Sequence:

Each lot of synthetic reagent must meet the standards in the filed Outline of Production of the product(s) in which it will be used. The standards should provide characterization and validation requirements for each lot of synthetic reagent and should include identity and purity standards appropriate for the intended uses of the reagent. The CVB may perform confirmatory testing of each lot of synthetic reagent.

VI. MISCELLANEOUS

Firms are encouraged to maintain a supply of approved MS and MCS, for access and use by APHIS for testing in the event of any unforeseen problem.

/s/ John R. Clifford

John R. Clifford
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