4.13 MASTER SEEDS/CELLS/SEQUENCES

1. Overview

The Master Seed and Master Cell concept is used in the manufacture of biological products. A Master Seed may be a bacterium, virus, or recombinant organism, e.g. a plasmid with an exogenous insert, expressed in an *E. coli* bacterial host. A Master lot of Seed or continuous Cell line is tested extensively for identity and purity by the firm, with confirmatory testing by the CVB laboratory. In addition, recombinant Master Seeds should be tested to ensure expression of the relevant antigen. Each Master lot must be approved by APHIS prior to use in the production of biological products. Once approved, all production must utilize seeds and cells that are direct passages from the Master lot. The maximum permissible number of passages from the Master lot is also defined. A single Master lot may be used in the manufacture of many different biological products.

Modern biotechnology enables manufacturers to use chemically synthesized ingredients in biological products as well. Some examples of these are primers in PCR test kits and synthetic peptides in antibody test kits. For these entities, a Master Sequence (amino acid, nucleotide, or carbohydrate) is specified in the Outline of Production; these may be commercially acquired and have Quality Control documents. The Master Sequences do not require confirmatory sequencing by CVB, although their suitability to detect disease-associated isolates or antibody to them should be evaluated. This can be done, e.g., through sequence alignment with GenBank or other database-stored sequences, through consultation with subject matter experts, or through published literature, and, as far as possible, testing with an appropriate panel of samples.

Other examples of Master Sequences include, but are not limited to, RNA vaccine sequences and therapeutic or prophylactic synthetic peptides. These injected biotechnology-derived biologics require confirmatory sequencing, as well as other tests for purity and identity.

2. References

The following regulations and guidance documents pertain to Master Seeds (MS) and Master Cell Stocks (MCS):

- 9CFR Parts 101.6, 101.7, 113.8, 113.27, 113.47, 113.52, 113.55, 113.64, 113.100, 113.200, 113.300
- Certain Seeds have additional requirements in product-specific sections of 9CFR Part 113.
- VS Memorandum 800.68: New Biotechnology for Preparation of Animal Biological Products (December 4, 1984)

- VS Memorandum 800.88: Testing for Reticuloendotheliosis Virus Contamination (August 23, 1999)
- VS Memorandum 800.89: Chicken Anemia Virus (December 22, 1999)
- VS Memorandum 800.109: Master Seed and Master Cell Stock Testing Report Submission
- VS Memorandum 800.113: Production, Testing and Storage of Master Seed and Master Cell Stocks at Alternate Locations (September 17, 2008)
- VS Memorandum 800.201: Backpassage Studies
- VS Memorandum 800.205: General Licensing Considerations: Biotechnology-derived Veterinary Biologics Categories I, II, and III
- Testing of Biological Products chapter of Reviewers' Manual
- LSRTIS Program Documentation

3. Flow of Information/ Office Procedures

3.1 Mail log entries: Submissions regarding MS and MCS should be entered into the mail log with a direct link to the applicable MS or MCS record in LSRTIS. This means that a MS or MCS record must be created when the first submission is received. Additionally, the submissions may be linked to the product code(s) in which they will be used, but this is optional.
When creating a MS or MC record in LSRTIS, ensure the Lot ID is *exactly and completely* as it appears on the MS or MC vial. Ask the firm for the complete identifier if they do not provide details of the vial labels in their MS/MC report.

Do not create LSRTIS records for "pre-master" seed submissions. These submissions can later be linked to the MS record for the master lot that is eventually produced, but pre-master submissions are simply listed as product code UNASGN at the time of their receipt.

3.2 Location of MS and MCS files:

3.2.1 MS and MCS documents are filed independently of products in which they are used. MS and MCS files are not archived unless the CVB receives notification from the firm that the MS or MCS no longer will be used in production.

3.2.2 *Unapproved* MS and MCS documents were historically filed in GREEN folders and placed alphabetically in the prelicense files. These historic files are located behind the product prelicense files for the establishment.

The folders were identified with the MS/MCS name *as it will be configured in LSRTIS* (example: Coronavirus, chicken NOT Infectious bronchitis virus) and the complete lot number. Master Sequences will not be entered into LSRTIS. Conformance with LSRTIS format provides a standard format to ensure that "like" Master SC are filed together.

3.2.3 *Approved* Master Seeds and Master Cells were historically transferred to BLUE folders and filed alphabetically in the Seed/Cell section of the power files for the establishment. MS and MCS data were entered into LSRTIS at the time of approval. When the reviewer writes the letter that the MS/MC has been approved (or it is unsatisfactory for use), the MS/MC LSRTIS record should be updated. Update Disposition from pending to Approved or Disapproved. If disposition is approved, update the Status (regarding inventory) from pending to Active Inventory. Add approved species and approval date. If disposition unapproved, change status to Deleted (as the inventory will not be used).

Master Sequences will not be entered into LSRTIS. See the LSRTIS Program Documentation for details. A yellow dot on the folder label indicates that LSRTIS entry has occurred.

3.1 CVB correspondence format:

3.3.1 CVB correspondence addressing MS or MCS issues should contain the following information in the first paragraph of the letter:

"This letter is response to your submission dated XXXX regarding <name of MS/MCS>, Lot <lot number of MS/MCS>, (<approved or unapproved>)."

[redacted]

Limit the first paragraph to one sentence, listing the Master Seed, to facilitate appropriate filing.

Do NOT list a product code in the first paragraph, even though the firm may submit MS/MCS information in connection with a product code. Doing so may create difficulties finding the submission in the mail log in the future. If you feel it is necessary to reference a product code as well, always list the MS first.

3.1 Be consistent in how the lot number for the MS/MCS is written in correspondence. (If the lot number varies, even slightly, from submission to submission, the support staff does not know if the various submissions pertain to the same Master SC.) Write the lot number *exactly* as it is written on the actual vial of Master SC and in LSRTIS records. Do not use abbreviations. If the firm does not specify how the vial is identified, ask the firm. (Be aware firms will often use slang/lingo rather than full identifiers on their submissions.)

3.2 If the MS/MCS has not yet received final approval for use in the production of biological products, the status in the first paragraph is "unapproved."

3.3 Write responses to MS/MCS issues separately from those associated with particular products. If the two topics are combined into one letter, it will make it more difficult to find pertinent information related to MS/MCS in the mail log.
3.4 If several MS and MCS are processed at the same time, ideally there should be a separate letter for each. This is the easiest way for the support staff to keep the files correct, and this will facilitate retrieval of the information from the mail log in the future.

4. Procedure for Approving a Master Seed or Cell

4.1 Ideally, the Master Seed or Cell should not be introduced into production facilities until it is approved by the CVB. Some firms, however, need to introduce a candidate Master Seed or Cell into production facilities in order to prepare the Master lot or to test it. If a firm makes a request to introduce an unapproved Master Seed or Cell into production, the reviewer should determine whether the unapproved seed/cell poses a material risk to the integrity of other production processes occurring in the facility. The reviewer should also consult with IC, as per ICSOP0018 to ensure that the new fraction can be introduced into the licensed premises with no disruption of the current production procedures and without cross contamination of products. Research facilities that are entirely separate and apart from facilities used for the preparation of licensed biological products will not be considered part of the licensed premises for these purposes. If not, permission may be granted to introduce the Master Seed or Cell into production facilities *at the firm's own risk*. Letters written to authorize moving the Seed into production should include a statement to update facility documents as necessary.

4.2 The firm must submit an acceptable report of their evaluation of the Master Seed or MCS candidate. Although an APHIS Form 2008 may be used to summarize test results, **a 2008 is not required and is NOT sufficient** to document the firm's testing. A complete report, with materials and methods, should be submitted, per VS Memorandum 800.109.

All tests codified in the 9CFR, plus any additional tests deemed necessary to assess the identity and purity adequately, must be completed. Specialized testing that the firm is not equipped to perform may be completed by outside laboratories.

The two key areas of testing are identity and purity. Identity testing of Master Seeds should adequately define the taxonomic type of the agent (e.g., bacteria to the species level in most cases) and should confirm any special characteristics of the agent (e.g., pilus or toxin expression, special serotype (e.g., *E. coli* O157:H7), or mutation (e.g., J-5 *E. coli*).

Purity testing for bacterial agents is relatively simple (9CFR 113.27) because they are grown in acellular medium in which contaminating viruses will not survive. Purity testing for viruses and other intracellular organisms, as well as cells, is more complex. Viruses, other obligate intracellular organisms, and cells must be tested for different

extraneous agents, depending on the species of animal from which they were derived, how they will be propagated during production, and in what type of biological product they will be used. For example, a feline virus, propagated on canine cells, for use in a feline vaccine must be tested for feline *and* canine extraneous agents. Some unique considerations for testing fish Master Seed Viruses include:

- When testing fish Master Seed Viruses, fish red blood cells should be used to testing for hemadsorbing agents as per 9 CFR 113.46(b).
- When testing aquatic Master Seed Viruses for cytopathic agents as per 9 CFR 113.55(c), fish cell lines should be maintained for at least 28 days.

The standard list of extraneous agents, divided by animal species, is found in 9CFR 113.47, but testing for additional agents may be required in individual cases, depending on risk factors. Some specific considerations for unique cell lines are listed below:

- Considerations for Avian cell lines
    - Agents of concern regarding chicken derived cell lines:
        - Reticuloendotheliosis virus (REV), and Chicken Anemia Virus (CAV): PCR tests are available for these viruses and protocols are available from the CVB laboratory.
        - Avian leukosis virus (ALV): An ELISA test is available, and the protocol is available from the CVB laboratory. To use this test, an exemption to 9 CFR 113.31 must be requested. The exemption request should be accompanied by data to support that the ELISA test conducted by the firm is at least as sensitive as the codified test.
        - Marek's disease virus (MDV): Very important because many of the poultry cell lines have been immortalized via MDV infection. Papers such as "Transactivation of Latent Marek's Disease Herpesvirus Genes in QT35, a Quail Fibroblast Cell Line, by Herpesvirus of Turkeys," by T. Yamaguchi et al in Journal of Virology 74:10176-10186 may be helpful.
    - Agents of concern regarding duck cells
        - Duck hepatitis virus: Should be detected in 9 CFR 113.37 testing
        - Duck enterovirus: This virus causes CPE in DEF cells
        - Duck parvovirus: Only grows in Muscovy duck fibroblasts and embryo fibroblasts
- Agents of concern for fish cell lines
    - Viral Hemorrhagic Septicemia Virus (VHS)
    - Infectious Hematopoietic Necrosis Virus (IHN)
    - Spring Viremia of Carp Virus (SVC)
    - Infectious Salmon Anemia Virus (ISA)
    - Infectious Pancreatic Necrosis Virus (IPN)
    - Channel Catfish Virus (CCV)

When technology advances to improve the sensitivity of confirmatory testing, this technology should be incorporated to ensure purity of the MS. For example, PCR techniques and Next Generation Sequencing are currently available to help confirm that extraneous BVD viruses are not present in BVD MS [redacted]

If a Master Seed is derived from recombinant technology, then a Summary Information Format (SIF) document is required; see VS Memorandum 800.205 and the SIF Chapter of the Reviewers' Manual for additional detail. SIFs are filed with the Risk Manager.

In addition, supporting studies like environmental stability, shed/spread, and safety testing in non-target species may be required for recombinant Master Seeds. These studies are summarized in the SIF. These supporting study submissions, and reviewer response, however, should be filed in the recombinant MS file.

When reviewing recombinant Master Seeds, be aware that use of any antibiotic resistance marker is currently strongly discouraged as a selection method unless it is removed or inactivated during manufacture of the final product. For example, if the bacterial host is inactivated, the plasmid and resistance gene (with a prokaryotic promoter) will also be inactivated. For some historical perspective, consider that kanamycin resistance used to be considered a useful selection marker for recombinant plasmids, because kanamycin was not used in human and animal medicine due to serious toxicity concerns. This has now changed and kanamycin may be used (though infrequently) for serious bacterial infections in which other antibiotics may not work.

4.3 If the firm's test report (and preliminary SIF, if applicable) is acceptable in format and content, then the reviewer may initiate authorization for submission of the Master Seed or MCS for confirmatory testing. [redacted]

4.3.1 If the Master Seed is derived from recombinant technology, approval from the CVB's Institutional Biosafety Committee (IBC) must be obtained before the CVB can accept samples of the Seed. The risk manager is responsible for obtaining this approval and providing notification, and the IBC number, back to the reviewer. See the SIF Chapter of the Reviewers' Manual for details.

[redacted]

4.3.3 Write a letter to the firm to authorize testing. Use the test authorization template letter in Appendix 3 of Reviewer Manual Chapter 5.1 Requesting Tests at the CVB Lab. If the firm used an APHIS Form 2070 to request permission to submit samples of the MS or MCS for confirmatory testing, you may simply include the test authorization number and number of vials needed to complete confirmatory testing on the form, sign, and return the form to the submitter.

4.3.3.1 Notify them of the test authorization number ("special request number"). Instruct them to include this number in the Remarks section of the APHIS Form 2020 that accompanies the shipment of samples.

4.3.3.2 Most Master Seeds are subject to shipping regulations for animal pathogens. **Select agents have additional rules.** It is the responsibility of the reviewer (in conjunction with laboratory personnel) to ensure all applicable preparatory steps have been taken to receive pathogenic organisms at the laboratory.

4.3.3.3 The laboratory coordinator will notify the reviewer of how many vials of MS or MCS that will be needed to complete confirmatory testing. In general, 18 vials are needed to complete confirmatory testing of recombinant MS.

4. [redacted]

4.5 [redacted]
Write letters formally approving (or denying) a MS/MCS for use in the production of biological products. [redacted]

[redacted]

[redacted]

4.7 [redacted]

5. Transfer of Master Seeds and Cells between Firms (Buy-outs, sub-licensing transfers)

It is the responsibility of the reviewer during a buy-out to determine which MS and MCS files need to be relabeled with the new establishment code and transferred to the new firm. Archive any files pertaining to MS or MCS that will not be used by the purchasing firm. [redacted]

6. Preparation of new "Master" lots from approved Seeds and Cells

Firms may run low on inventory of the original Master lot of a Seed or Cell. They may then elect to prepare a new lot (often an X+1 passage) to use as a new Master lot. The current MS/MC record should be updated to indicate the inventory has been "Depleted".

6.1 The firm should submit a seed/cell report per VS Memorandum 800.109 for the new master lot. A new record should be created in LSRTIS.

6.2 The new master lot should undergo confirmatory testing by the CVB. Ensure all documentation provides a clear link between the new lot and the original Master lot.

6.3 Historically it was practice to "refresh" certain Master Seeds by occasional passage through animals. This was especially common with *Leptospira* and

certain *Clostridium* species. This is no longer acceptable practice! Once a Seed has been passed through animals, it is subject to all requirements of a new Master Seed. [redacted]

7. Archival of Obsolete/Depleted Master Seeds and Master Cell Stocks

7.1 If a firm notifies the CVB that a particular Master Seed or MCS has been depleted, destroyed, or otherwise will not be used anymore in production, [redacted]

8. Seeds/Cells used in manufacture of FDA-EREA products

8.1 Seeds and Cells used exclusively in the manufacture of products exported under FDA-EREA must be tested by the firm before they can be introduced into production facilities. Reviewers may elect to allow firms to move such Seeds and Cells into production *at the firm's own risk*.

8.2 Seeds and Cells used exclusively for FDA-EREA purposes are not generally tested by the CVB. [redacted]

9. Extra requirements for Master Seeds/Cells used in the manufacture of live/modified live vaccines

9.1 Master Seeds to be used in the manufacture of live or modified live vaccines must undergo backpassage (reversion-to-virulence) and shed/spread studies(See VS Memorandum 800.201). Other potential studies include, but are not limited to, environmental stability and safety in non-target species. Final approval of the Seed

for use in the production of live biological products should not be granted until acceptable supporting studies have been reviewed.

9.2 A SIF template exists for conventional live vaccines. The CVB has not typically required its use, but the reviewer, in conjunction with the risk manager, may require it if individual circumstances warrant it. A copy of the SIF is filed in the Master Seed file (another copy is filed in the Biotech MS file for biotech products).

9.3 QT35 (quail tumor) cells are generally recognized to have contamination with latent Marek's Disease virus. Thus, they are NOT to be used in the production of live/modified live vaccines!

10. Requirements for Human Master Cell Stocks

10.1 At least one Human cell stock has been approved for use in animal biologics [redacted]

10.2 Identity testing of Human cell stocks is necessary, [redacted] Confirmatory testing includes testing the MCS against species identification stains for bovine, canine, equine, feline, ovine, porcine, and vero cells. All results should be negative.

10.3 Karyology, mycoplasma, and sterility testing should be conducted.

10.4 If the cell line is grown in bovine or equine serum, the MCS should be tested for bovine and equine viruses, as well as the standard panel of Bluetongue Virus, Reovirus, and Rabies virus. Cell lines used to test for cytopathic and hemadsorbing agents should include human cell lines, and a cell line representing the species of animal the final product will be used in. Equine and bovine cell lines should also be used to test for cytopathic and hemadsorbing agents if equine or bovine serum was used to grow the MCS.

10.5 Samples of the human cell line should be sent to the Foreign Animal Disease Diagnostic Lab at Plum Island and tested by pan viral microarray for human extraneous agents.

11. Expanded approvals for Master Seeds and Cells subsequent to initial approval

Occasionally, additional testing will be performed on a Master Seed or Cell subsequent to its initial approval for use in production. Most commonly this occurs when additional extraneous agent testing is required to obtain an additional animal species approval.

11.1 Correspondence relating to the additional testing should be formatted to show the Seed or Cell is *approved*, even though the expanded approval has not yet been given.

11.2 When the expanded approval is given, update the LSRTIS record as appropriate.

[redacted]