

**United States Department of Agriculture
Center for Veterinary Biologics**

Standard Operating Policy/Procedure

Master Seed Testing in the Virology Section

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Master Seed Testing in the Virology Section

1. Purpose/Scope

This document outlines the procedures for evaluating conventional and recombinant viral and other obligate intracellular or protozoal Master Seed(s) (MS) in the Center for Veterinary Biologics (CVB) Virology Section. The main emphasis of this document is to describe testing of MS for extraneous agents. Unless otherwise prescribed in a Standard Requirement, each MS submitted to the CVB must be tested for extraneous agents in accordance with title 9, *Code of Federal Regulations* (9 CFR), section 113.55.

Purity, safety, identity, and avian origin MS requirements are covered in 9 CFR 113.300 and applicable Veterinary Services (VS) Memorandums.

The MS must be tested for sterility and Mycoplasma in accordance with 9 CFR 113.27 and 113.28, respectively. In addition, testing for Salmonella contamination is required for avian origin MS and live vaccines as described in 9 CFR 113.30. These tests are conducted by the CVB Bacteriology Section, and Virology staff should coordinate use of samples with personnel from that Section as necessary.

2. Prerequisites for Master Seed Extraneous Agent Testing

Personnel must be experienced in general virological laboratory techniques.

Technician staff and the Veterinary Medical Officer/Microbiologist (VMO/Micro) agent contact should meet and plan testing strategy for each MS presented for testing.

Technician staff must keep the VMO/Micro agent contact informed of testing progress and provide updates as agreed upon during the planning phase.

2.1 Master Seed historical information

Prior to initiating the testing of a MS, obtain as much information as possible about the origin, passage history (including purification and characterization procedures), and storage conditions for the test MS. This information must be provided by the biologics manufacturer to the CVB Review Staff in the master seed test report in accordance with VS Memorandum No. 800.109. In addition to testing results, the following items should be included in the MS test report:

- a. Seed origin and passage history
- b. Identity
- c. Purity
- d. Extraneous agents
- e. Summary information formats
- f. Addenda
- g. Passage level for testing MS

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The material used for MS testing must be the MS, unless otherwise approved by the CVB.

2.2 Substrate purity requirements

Any cells used in MS testing must be satisfactory when tested as prescribed in 9 CFR 113.51 and 113.52. Embryonated eggs must also meet the purity and quality requirements as prescribed in VS Memorandum No. 800.65.

2.3 Testing of Master Seed consisting of persistently infected master cell stock

When the MS consists of a persistently infected master cells stock (MCS), the tests shall be carried out on MCS lysate.

3. Testing Procedures for Detection of Extraneous Agents in Master Seeds

Refer to the 9 CFR and current versions of specific testing protocols (PROs), supplemental assay methods (SAMs), and standard operating policies/procedures (SOPs) for performing extraneous agent testing on MS. All information pertaining to MS testing must be recorded on **VIRTWS2001**, *Master Seed Virus Extraneous Testing – Tested According to VIRSOP2007*.

3.1 Neutralization of Master Seed

The neutralization of MS is performed in compliance with 9 CFR 113.55 following the procedures described in the current version of **VIRPRO1013**, *Neutralization and Passage of Master Seed in Cell Cultures*. If the MS is capable of replicating in the cell or culture system in which it is to be tested, the MS must be neutralized (monospecific antiserum, monoclonal antibody, or alternative method) prior to testing for extraneous agents. In the event the CVB is unable to neutralize the test MS with the CVB's antiserum, the biologics manufacturer is required to provide a sufficient amount of neutralizing material to perform the extraneous agent testing of the MS and to confirm the specificity of the neutralizing material itself.

3.2 Passage of Master Seed in permissive cells or culture system

The passage of MS in cells or culture systems is performed in compliance with 9 CFR 113.55 following the procedures described in **VIRPRO1013**. Briefly, MS should be subcultured in cells or culture systems (embryonated eggs, laboratory animals, etc.) which are permissive to extraneous agents of interest for 14 days. The 9 CFR 113.55

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requires the following cell cultures be used for testing of all MS that have been in contact with mammalian tissues or cells:

- a. African green monkey kidney (Vero);
- b. Cell cultures derived from fetuses, neonatal cells, or a cell line of the species for which the vaccine is recommended;
- c. Fetal cells, neonatal cells, or a cell line of the species of cells in which the MS has been propagated if different than the one given in (a) and (b) above; or
- d. Fetal, neonatal, or cell line of species in which the pre-MS previously was isolated and/or propagated (if different than a, b, or c).

Information provided in the MS report by the biologics manufacturer will assist the CVB in determining the type of cells or culture system needed for testing in addition to the 9 CFR required cell cultures. When developing the appropriate testing strategy for a given MS, consideration should be given to the use of primary cells in addition to cell lines.

3.3 Detection of extraneous agents by fluorescent antibody (FA) staining

The detection of extraneous viruses by FA staining (direct/indirect) is performed in compliance with 9 CFR 113.47 following the procedures described in the current version of **VIRPRO1014**, *Fluorescence Antibody Staining Procedure for Detection of Viral Antigens*. Briefly, at the last subculture of the MS inoculated cells, seed appropriate vessels (chambered slides or other suitable vessel) with MS inoculated cells. Seed uninoculated cells for preparation of controls for each cell line. For preparation of positive controls, inoculate cells with an appropriate concentration of positive control virus following seeding of vessel (co-inoculation). Seven days after the last subculturing, fix and stain MS inoculated and control monolayers. To ensure or enhance fluorescence detection, additional MS and positive control monolayers may be fixed before Day 7. Regardless of when monolayers are fixed, they must be stained concurrently.

For rabies virus, stain a CVB previously prepared positive control slide.

3.4 Detection of hemadsorbing agents

The detection of extraneous viruses by hemadsorption (HAd) is performed in compliance with 9 CFR 113.46 following the procedures described in the current version of **SAM 313**, *Supplemental Assay Method for the Detection of Extraneous Hemadsorbing Agents in Master Seed Virus*. Briefly, cell monolayers in 25-cm² flasks containing MS are overlaid with chicken and guinea pig red blood cells (RBC) and incubated at 4°C and 25°C consecutively. Negative controls should be included for each cell line. A positive control must be included on a cell monolayer to validate the assay.

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3.5 Detection of viral intracellular inclusions and related cellular changes by the hematoxylin and eosin staining method

The detection of extraneous viruses by histological staining (hematoxylin and eosin, H-E) is performed in compliance with 9 CFR 113.46 following the procedures described in the current version of **VIRPRO1012**, *Hematoxylin and Eosin Staining Procedure for Detection of Cytopathic Extraneous Agents in Master Seeds*. This modified H-E staining detects inclusions and other cellular changes induced by viral infections in cultured cells. Briefly, at the last subculture of the MS inoculated cells chambered slides are seeded. Positive and negative controls should be prepared for each species tested. One positive chambered slide is inoculated with a virus known to induce intra-cytoplasmic inclusions and one slide is inoculated with a virus known to induce intra-nuclear inclusions.

3.6 Detection of Seneca Virus A (SVA)

The detection of extraneous SVA is performed following procedures described in the current version of **VIRPRO0129**, *Amplification of Extraneous Senecavirus Through Cell Culture*. Briefly, a cell culture assay, with Real time PCR and/or IFA methods to detect the presence of SVA in MS performed after last subculture has been through 3 freeze/thaws cycles.

4. Testing Procedures for Detection of Extraneous Agents in Master Seeds Originated or Passaged in Chicken Cells/Embryos

4.1 Detection of avian lymphoid leukosis

The detection of extraneous avian leukosis virus is performed in compliance with 9 CFR 113.31 following the procedures described in the current version of **VIRPRO0415**, *Detecting Extraneous Avian Leukosis Virus in Biologic Products by p27 ELISA*. Briefly, MS which are cytopathic to chick embryo fibroblast (CEF) cells, shall be effectively neutralized (monospecific antiserum, monoclonal antibody, or alternative method) and inoculated onto secondary CEF cells. Positive and negative controls should be tested on CEF cultures. The cell cultures shall be passed when necessary to maintain viability for at least 21 days. Harvested materials from each passage will be stored at -60°C or colder until tested for group specific antigen by enzyme-linked immunosorbent assay (ELISA).

4.2 Detection of hemagglutinating viruses

The detection of hemagglutinating viruses is performed in compliance with 9 CFR 113.34 following the procedures described in the current version of **VIRPRO0096**, *Detection of Hemagglutinating Viruses*. Briefly, MS which cause hemagglutination, shall be effectively neutralized (monospecific antiserum, monoclonal antibody, or alternative method) and inoculated via the allantoic route into 9- to 11-day-old embryonated eggs. Following incubation for 4 to 5 days, amnio-allantoic fluid (AAF) is harvested from eggs

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for testing. Testing is performed by mixing a 0.5% chicken RBC suspension with the AAF sample. Newcastle Disease virus antigen will be used as a positive control. Wells with complete hemagglutination are positive. Wells with distinct button formation are negative. Samples which test positive will be further characterized by the hemagglutination-inhibition test.

4.3 Detection of reticuloendotheliosis virus (REV)

The detection of extraneous REV is performed in compliance with VS Memorandum No. 800.88 following the procedures described in the current version of **VIRPRO0119**, *Polymerase Chain Reaction Assay for Detection and Identity of Extraneous Reticuloendotheliosis Virus (REV)*. Briefly, a polymerase chain reaction (PCR) assay using REV specific primers is used to detect extraneous REV in MS. MS are passed through tissue culture prior to DNA extraction. Other acceptable methods for REV detection, in addition to PCR, are outlined in the Memorandum.

4.4 Detection of chicken anemia virus (CAV)

The detection of extraneous CAV is performed in compliance with VS Memorandum 800.89 following the procedures described in the current version of **VIRPRO0118**, *Polymerase Chain Reaction Assay for Detection and Identity of Extraneous Chicken Anemia Virus (CAV) DNA*. Briefly, a PCR assay using CAV specific primers is used to detect extraneous CAV in MS. Other acceptable methods for CAV detection, in addition to PCR, are outlined in the Memorandum.

5 Additional Master Seed Testing Requirements

5.1 Identity testing

Identity testing is performed in compliance with 9 CFR 113.300. To confirm identity, the MS should be tested by fluorescent antibody test, serum neutralization test, or by an alternative method approved by the Virology agent contact.

5.2 Safety

Safety testing is performed in compliance with 9 CFR 113.300. Briefly, MS recommended for animals other than poultry shall be tested for safety in at least one species for which the vaccine is intended using methods prescribed in 9 CFR 113.39, 113.40, 113.41, 113.44, and 113.45 or in a filed Outline of Production. Poultry MS shall be tested as prescribed in 9 CFR 113.36 and 113.37.

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6. References

Title 9, *Code of Federal Regulations*, sections 113.31, 113.34, 113.46, 113.47, 113.55, 113.300, 113.309, U.S. Government Printing Office, Washington, DC.

7. Summary of Revisions

Version .04

- **3.6:** addition of Seneca Virus A testing.

Version .03

- The Contact information has been updated; however, the Virology Section has elected to keep the same next review date for the document.
- **2:3:** Deleted reference to Agent Biosecurity and Reference Management Section.

Version .02

- The Contact information has been updated.