



Animal and Plant
Health Inspection
Service

Veterinary Services

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March 19, 2014

VETERINARY SERVICES MEMORANDUM NO. 800.119

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

FROM: John R. Clifford /s/ *John R. Clifford*
Deputy Administrator

SUBJECT: Exemptions to title 9, *Code of Federal Regulations* (9 CFR), part 113.28, Detection of Mycoplasma Contamination

I. PURPOSE

This memorandum provides guidance to licensees, permittees, and applicants for obtaining an exemption from the Center for Veterinary Biologics (CVB) to conduct mycoplasma contamination testing, using a protocol harmonized under the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) guidelines.

II. BACKGROUND

In the United States, the present method for conducting mycoplasma contamination testing is codified in 9 CFR 113.28. It requires using a culture technique of broth enrichment and agar detection. The test is required for Master Seed Virus lots, Master Cell lots, and final product testing, as well as for ingredients of animal origin that are not subjected to heat sterilization. In terms of final product testing, all serials of live viral products are tested. For inactivated product, if the inactivating agent is shown to inactivate mycoplasma, serials of that product are exempted from the test. Details on this exemption can be found in Veterinary Services (VS) Memorandum No. 800.86, entitled, "Exemption from Mycoplasma Testing Under 9 CFR 113.200(c)(3)." The exemption does not allow contamination but acknowledges the test result is meaningless.

The VICH is working to harmonize testing and licensing of new products for veterinary use so that a unified standard may produce mutually acceptable data for regulatory authorities. This process has produced harmonized testing procedures for the determination of residual-free formaldehyde content and the determination of moisture content in desiccated products. The harmonization of testing for mycoplasma contamination is addressed in the VICH GL34 document, "Testing for

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the Detection of Mycoplasma Contamination.” It should be noted that GL34 requires specific tests, as well as information on when these tests are utilized. The GL34 guidelines are found at http://www.vichsec.org/en/GL34_st7.doc.

Additional documents relative to this memorandum can be accessed from the CVB:

- SAM 910, entitled, “Supplemental Assay Method for Detection of *Mycoplasma* Contamination,” which outlines a broth and agar culture technique codified in 9 CFR 113.28.
- Protocol BBPRO1007, entitled, “Indicator Cell Culture Method for Detecting Mycoplasma Contamination.”

The VICH guideline describes two test methods and allows for a third:

- 1) Expansion in broth culture and detection by colony formation on nutrient agar plates, referred to as broth and agar culture.
- 2) Expansion in cell culture and characteristic fluorescent staining of deoxyribonucleic acid (DNA), referred to as DNA stain.
- 3) Polymerase chain reaction or nucleic acid amplification technique (PCR or NAT) is acknowledged and may be approved if adequately validated by the firm for equivalency with one or both tests designated above.

The broth and agar culture technique outlined in GL34 differs from the procedure outlined in 9 CFR 113.28/SAM 910. For example, in 9 CFR 113.28, a 0.1 mL sample is inoculated on the agar plates. In GL34, the inoculum volume is 0.2 mL. In 9 CFR 113.28, the broth cultures are incubated 14 days. In GL34, the broth culture is incubated for 20-21 days. In 9 CFR 113.28, agar plates are incubated in 5%CO₂ in air. In GL34, agar plates are incubated in 5%CO₂ in nitrogen.

The DNA stain procedure has no similar counterpart in U.S. regulations.

III. POLICY

Firms may elect to continue following the 9 CFR requirements by continuing to reference 9 CFR 113.28 in their Outlines of Production and Master Seed/Cell Reports.

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Firms electing to seek exemption to the 9 CFR requirements and follow a properly validated GL34 protocol should use the tests as follows:

Material	Broth and agar culture*	DNA Stain*
Master Seed virus	Required	Required
Master Cell Stock	Required	Required
Working and Production seed virus	Required	Required
Working and Production Cell stock	Required	Required
Ingredient of animal origin	Required	
Final Product	Required by the U.S.	

*refer to GL34 for protocol.

GL34 discusses testing harvest materials when testing is required. We encourage firms to test harvest materials but currently require final product testing. In some cases, both tests are required.

9 CFR 113.4 requires compliance with the test methods described in all applicable Standard Requirements unless otherwise exempted. Exemptions are to be noted in the filed Outline of Production. Guidance regarding the documentation of exemptions can be found in VS Memorandum No. 800.206.

Inactivated viral vaccines exempt from mycoplasma testing as described in VS Memorandum No. 800.86 will continue to be considered exempt from the testing.

For viral Master Seed testing, Master Cell testing, or confirmatory and check testing, our laboratory will utilize the test cited in the Outline of Production or Master Seed/Cell report.

V. IMPLEMENTATION/APPLICABILITY

Exemption requests should be made to the assigned reviewer. The requests should be accompanied by the following documents:

- Special Outline(s). This document(s) should provide the details of the tests, including media volumes and neutralization steps, and note that they are in accordance with GL34.
- Culture test system validation. Low levels of five strains of mycoplasma should be detected using the protocol in the Special Outline discussed above.
- Media nutritive properties. Each lot of the media used should be shown to support mycoplasma growth. Note that after the exemption is granted, this testing is conducted with each new lot of media, and a Special Outline should be filed to

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describe the testing. Refer to GL34 for the parameters for selection of lots of mycoplasma for validation and individual serial tests.

- Inhibitory substance testing. The nutritive test in the presence and absence of the test material should be conducted. Any inhibitory substances must be neutralized, or their effect will otherwise be countered by techniques such as dilution. The date that data were accepted to support the technique or media volume should be included in the relative Outlines of Production. Firms may group similar products for this testing.
- Vaccine Virus Neutralization. If the vaccine virus causes CPE in the cell culture system, it must be neutralized. The serum should be tested for any inhibitory effects on mycoplasma growth, and these results supplied with the exemption request.

Once granted, the exemption should be documented in the relevant Outline of Production or Master Seed/Cell Report, in accordance with VS Memorandum No. 800.206.

It is our intent to update 9 CFR 113.28 to be consistent with this memorandum and the VICH guideline.

Our laboratory is currently validating a PCR-based test. More information on the use of that test will be provided at a later date.