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United States Department of Agriculture

Animal and Plant Health Inspection Service

Veterinary Services

Center for Veterinary Biologics

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CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 12-06

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

FROM: Richard E. Hill, Jr. */s/ Richard E. Hill, Jr.*
Director
Center for Veterinary Biologics

SUBJECT: Detection of *Mycoplasma* Contamination in Vaccines and Biological Products

I. PURPOSE

The Center for Veterinary Biologics (CVB) is validating a Polymerase Chain Reaction (PCR) detection assay as an alternative for the agar culture method specified in Title 9, Code of Federal Regulations (9 CFR), Part 113.28, Detection of Mycoplasma Contamination, and is planning a field study as part of the validation.

II. BACKGROUND

Veterinary biologics, viral master seeds, master cell stocks, and ingredients of animal origin used in the production of veterinary biologics must be shown to be free of contaminating mycoplasma, following the procedure specified in 9 CFR 113.28. This test involves a broth culture step for the amplification of low levels of viable mycoplasma, followed by an agar plating step for the detection of mycoplasma colonies. The test procedure differs from assays required by regulatory agencies of other countries; consequently, products exported to certain countries must be tested by both 9 CFR113.28 and by the method accepted by the importing country.

With the aim of harmonization of the general requirements for mycoplasma contamination testing, the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) has developed draft guideline GL34, “Testing for the detection of mycoplasma contamination”. This document provides for the option of using a validated nucleic acid amplification methodology, provided that the detection limit of the assay is at least equivalent to that of the culture methods listed in GL34. Upon approval of the draft guideline, the guideline will be recommended for adoption by the CVB.

III. ACTION

The CVB has developed a PCR assay for the detection of mycoplasma, and is currently in the process of validating the method for implementation at the CVB. The PCR assay does not replace the 9 CFR 113.28; it is intended for use with broth enrichment, replacing the agar detection step of 9 CFR 113.28. The CVB laboratory is currently using the PCR assay to screen viral Master Seeds and Master Cell Stocks, and is running the PCR assay in parallel with the 9 CFR 113.28 procedure for routine mycoplasma testing.

The CVB is soliciting help from firms willing to participate in a field study to evaluate the reproducibility of the PCR method. Participating firms will be asked to test the PCR detection method side-by-side with the 9 CFR 113.28 agar detection method, using the five mycoplasma strains that are listed in GL34. The CVB will provide a detailed study protocol and will supply the mycoplasma strains, PCR protocol, and positive control. Firms wishing to incorporate the PCR assay into their mycoplasma testing may use the data they generate in the field study as part of their validation of the assay.

IV. IMPLEMENTATION/ APPLICABILITY

Requests for additional information should be directed to Dr. Geetha Srinivas at Geetha.B.Srinivas@aphis.usda.gov.