November 27, 2000

CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 00-15

Subject: Issuance of New Supplemental Assay Method

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

The following Supplemental Assay Method (SAM) has been approved:

STSAM0910.01 Supplemental Assay Method for Detection of Mycoplasma Contamination. This SAM is new and describes the test procedure used to detect Mycoplasma contamination in live viral products, master cell stocks, and Master Seed Viruses. If Mycoplasma contamination is present, colonies will form on the agar as seen under a stereoscope. A draft of this SAM was circulated to CVB staff and biologics licensees, permittees, and applicants; the following comments were received. Changes made to the SAM, or additional information in response to the comment, are noted by comment.

Comment 1: **Section 2.1.3** specifies the use of a 30°-35° C incubator. The Code of Federal Regulations, Title 9, Part 113.28, states that one must use a 33°-37° C incubator.
Response 1: We accept this comment and have changed **Section 2.1.3** to read "33°-37° C incubator."

Comment 2: **Section 2.2.6** describes the use of "Glassware: tubes, flasks, petri dishes." This section should allow flexibility in the type of glassware used, e.g., "or equivalent" could be used to provide for the use of disposable plastics.
Response 2: We accept this comment and have deleted the word "Glassware."

Comment 3: A comment was received which questioned the intent and interpretation of some of the positive control testing specified in **Section 3.3.3**.
Response 3: Sections 3.3.3 and 3.3.4 describe the controls as they are tested at the CVB-L. The 9 CFR, Part 113.28 (d)(4) indicates that both positive control tests, with selected Mycoplasma cultures, and negative control tests should be conducted. The CVB-L has chosen to use *Acholeplasma laidlawii* as a species to test the growth promotion qualities of the broth by detecting dark precipitation of the color indicator at a set titer. *Mycoplasma hyorhinis* was chosen to test the growth-promoting qualities of both the broth and agar through detecting colony-forming units. The use of other strains of Mycoplasma as controls is acceptable if their use can adequately detect changes in the growth-promotion qualities of the media.

Comment 4: One comment requested clarification of the required order of sample inoculation, such as negative control, samples, and then positive control, in **Section 4.10**. The comment also requested the identity of the positive control and how it was prepared, if different from that described in **Section 3.3.3**.

Response 4: The 9 CFR, Part 113.28(d)(4) only requires that the controls are tested simultaneously with the detection test using techniques provided in paragraphs (d)(2) and (3). The CVB-L tests their controls after the firm's samples are inoculated. The controls are tested as stated in **Sections 3.3.3** and **3.3.4**. Each laboratory's situation and purpose for controls may be different.

Comment 5: One comment questioned the usefulness of the "Z" streak specified in **Section 4.11**. They felt it was not necessary and difficult to do when only inoculating 0.1 ml.
Response 5: The CVB-L believes that some sort of streak or mark on the agar surface is necessary to enable the technician to focus the stereoscope on the surface of the agar. Due to the variability in the depth of agar in plates, the Mycoplasma colonies can be missed if the technician is not focused in on the surface of the agar. The streak or mark may also be done on the day the plate is read rather than on the day of inoculation.

Comment 6: One comment pointed out that in **Section 4.15,** additional plates and VERO cells are inoculated. The comment points out that this is not required by the 9 CFR, Part 113.28.
Response 6: We agree with this comment. The purpose of **Section 4.15** is to inform the biologics industry of the extra testing being conducted at the CVB-L to assure that the seeds and cells used to produce U.S. vaccines are tested for those Mycoplasma contaminants that may require microaerophilic incubation conditions or are unable to grow on agar surfaces and require a cell substrate to be detected. Its inclusion in the SAM is intended to keep the U.S. veterinary biologics industry current with the world guidelines and the scientific literature on Mycoplasma detection. The CVB-L also intends to begin testing Master Seeds and cells with polymerase chain reaction to gather data on this test's acceptability for detecting Mycoplasma contamination.

Comments 7-10 referred to the use of specifics in **Sections 2.1.5, 2.2.7, 4.1, 4.3, 4.9, 5.1, 8.1, and 8.2**. We agree that other methods of disinfection, other lab wear, other media production facilities, and other days between 10-14 to read the agar plates are acceptable for use in other laboratories. This SAM is a guideline for the performance of the test at the CVB-L.

This SAM is also available as an Adobe Acrobat pdf file on the world wide web (WWW) at http://www.aphis.usda.gov/vs/cvb/lab.

For those firms and interested parties with E-mail addresses and WWW access to the SAMs, this notification has been sent via electronic mail. If you would prefer to receive information from the Center for Veterinary Biologics via electronic mail, please send your E-mail address to cvb@usda.gov.

/s/ Randall L. Levings

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