



Animal and Plant
Health Inspection
Service

Veterinary Services

1400 Independence
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Washington, DC
20250

June 27, 2014

VETERINARY SERVICES MEMORANDUM NO.800.120

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

FROM: John R. Clifford /s/ *Jack A. Shere, for*
Deputy Administrator

SUBJECT: Dilution of Preservative Screening for Sterility Testing of Veterinary
Biologics

I. PURPOSE

This memorandum provides guidance on sterility testing of veterinary biological products. It is meant to assist licensees, permittees, and applicants in meeting sterility requirements in *title 9, Code of Federal Regulations (9 CFR)*, part 113 sections 113.25(d), 113.26, and 113.27.

II. REPLACEMENT

This memorandum combines information that was originally provided in the following Center for Veterinary Biologics (CVB) Notices:

- CVB Notice 09-02 - "Dilution of Preservative Screening for Broth-based Sterility Tests," dated January 22, 2009.
- CVB Notice 09-25 - "Dilution of Preservative Screening for Plate-Based Sterility Tests," dated December 31, 2009.
- CVB Notice 12-21 - "Conducting Dilution of Preservative Studies for Live Bacterial Vaccines," dated October 15, 2012.

III. BACKGROUND

Sterility of veterinary biologics is determined in direct inoculation tests codified in 9 CFR 113.26 and 113.27. Manufacturers are required, under 9 CFR 113.25(d), to determine the ratio of inoculum to medium, which shall result in sufficient dilution of such product to prevent bacteriostatic and fungistatic activity. Currently, live bacterial products without antibiotics or oral products may be granted an exemption to 9 CFR 113.25(d) upon request.

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The amount and type of media found acceptable at each temperature and the date these dilution of preservative data were accepted, or the date a product exemption was given are recorded in section V.A. of the Outline of Production.

The following table provides references to our laboratory procedures for the above codified testing:

Material tested	9 CFR*	Supplemental Assay Method (SAM)*
Killed biologics	113.26	906
Master Seed viruses	113.27(c)	908
Live Viral biologics	113.27(a)	
Live poultry biologics not for injection	113.27(e)	909
Master Seed bacteria	113.27(b)	928
Live bacterial biologics	113.27(d)	
Dilution of preservative	113.25(d)	903

*The current version of the document

Since 2007, the CVB has been screening the media volumes indicated in the Outlines of Production for dilution of preservatives as serials were picked for check or confirmatory sterility testing. The screening test is the inoculation of a single extra tube or plate (11th vessel) with indicator organisms and the serial. The extra vessel is observed for growth, with the lack of growth being suggestive of product inhibition/interference. A dilution of preservative test is performed in accordance with the appropriate SAM listed above. The data from 2007 and 2008 were combined and provided to the industry in 2009 by CVB Notices 09-02 and 09-25. For the broth-based tests, the data suggested that for 7% of the serials tested at the CVB, the dilution of preservative study may not have provided an adequate dilution to result in a valid test. For the plate-based test, the data suggested that for 35% of the serials tested at the CVB, the dilution of preservative study may not have provided an adequate dilution to result in a valid test.

Serials of licensed product that do not pass the full dilution of preservative testing, in accordance with SAM 903, are reported by the CVB as a no test for the sterility test and unsatisfactory for dilution of preservative. The APHIS Form 2008 is then processed as not to be released with a copy of the CVB Test Report provided.

For prelicensing serials, the firm is informed of the result by correspondence from the assigned reviewer with the associated CVB Test Report enclosed. No license is issued until the matter is resolved.

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IV. POLICY

Since insufficient media volumes to produce valid sterility tests continue to be found in our laboratory, the policy (as first announced in the notices) will continue as a standard practice.

In some cases, past history of the product or other suggestive evidence may result in testing according to 9 CFR 113.25(d)/SAM 903, as well as/or instead of the eleventh vessel screening test.

V. IMPLEMENTATION/ APPLICABILITY

The eleventh vessel screening test and subsequent SAM 903 testing and regulatory actions will continue as originally implemented. Therefore, that aspect of the memorandum should be considered as implemented immediately. Since factors, in addition to the eleventh vessel, have been noted, this policy should be considered as implemented 30 days from publication of this memorandum.