



December 31, 2009

CENTER FOR VETERINARY BIOLOGICS NOTICE NO. 09-25

**United States
Department of
Agriculture**

Animal and Plant
Health Inspection
Service

Veterinary Services

Center for Veterinary
Biologics

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

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

SUBJECT: Dilution of Preservative Screening for Plate-Based Sterility Tests

I. PURPOSE

The purpose of this Notice is to inform interested parties that the Center for Veterinary Biologics (CVB) is conducting a screening test regarding dilution of preservative in accordance with the Title 9, Code of Federal Regulations (9 CFR), part 113.25(d) for sterility testing using the method outlined in 9 CFR 113.27(e) and Supplemental Assay Method (SAM) 909. Additional plates at each temperature, with the volume of media indicated in the Outline of Production, are inoculated with the serial being confirmatory or check tested, and approximately 100 colony-forming units of the appropriate indicator organism (*Bacillus subtilis* or *Candida krusei*). If a reduced number of colonies occur on the plates with the serial, the CVB will test the dilution of preservative information for the product code utilizing the serial in question.

II. BACKGROUND

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. The CVB has been screening the media amounts as serials are picked for check or confirmatory testing by inoculating extra plates with indicator organism (*B. subtilis* at 30-35°C and *C. krusei* at 20-25°C) and the serial. For Activity Year 2007 (September 1, 2006 to August 31, 2007) and part of Activity Year 2008 (September 1, 2007 to January 10, 2008), the following results were obtained:

Activity Year	Number of Tests		
	9 CFR 113.27(e) Total tests	No. of tests with 20-25°C interference	No. of tests with 30-35°C interference
2007	40	1	12
2008	15	0	6
Total	55	1	18



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The CVB has previously determined that the plating error for this testing is +/-20% in our laboratory. Therefore, those tests showing reduction of equal to or greater than 20% were tabulated as showing interference. The range of interference seen for *B. subtilis* in 2007, ranged from 22-63%. In 2008, it is currently ranging from 22-70%. For *C. krusei* the interference was a 20% reduction in colony-forming units. The above data suggests that for 35% of serials tested at CVB, the dilution of preservative study may not have provided an adequate dilution of preservative to result in a valid test.

III. ACTION

The CVB will begin repeating dilution of preservative studies (utilizing the volume of media indicated in the Outline of Production) per 9 CFR 113.25(d) and the current version of SAM 903 when the above screening test suggests that the media volume being used is insufficient. This action will be initiated 30 days after the publication of this Notice. This will add approximately 3 weeks to serial testing for those serials slated for further testing. If the CVB's dilution of preservative test indicates interference at the volume cited in the Outline of Production, the CVB testing will be reported as an NT (no test) result for the sterility testing and a U (unsatisfactory) result for the dilution of preservative test. The APHIS Form 2008 will be processed as NRL (not to be released) - the serial will not be marketed.

In the case of confirmatory testing conducted during the licensure process, no license will be issued until the dilution of preservative testing is reevaluated and the issue of media volume is resolved. In the case of check testing for a licensed product, the serial will be reported out as unsatisfactory. Further regulatory action will be taken as appropriate until the issue of media volume is resolved.