

**United States Department of Agriculture  
Animal and Plant Health Inspection Service  
Center for Veterinary Biologics  
P. O. Box 844  
Ames, IA 50010**

1. **Reagent Name:** *Acholeplasma laidlawii*
2. **Strain or Source:** BRP batch 1; European Directorate for the Quality of Medicines & HealthCare (EDQM); Strasbourg, France.
3. **Lot Number:** 17Apr12-AL
4. **Fill Date:** April 17, 2012
5. **Expiration Date:** None

**Precautions:** This reagent must be handled in a manner consistent with Biosafety Level 2 (BSL-2) standards.

6. **Intended Use:** For use as a reference standard in the culture method to determine mycoplasma contamination of biological products.
7. **Instructions for Use:** Vials of the culture should be rapidly thawed in a 35°-38°C water bath and immediately diluted in sterile saline (0.8-0.9% NaCl solution) to achieve the desired concentration of colony forming units (CFU); refer to Section 14. To maintain viability, culture dilutions must be inoculated onto medium within 10 minutes of preparation.
8. **Test of Reagent:** Purity of this reagent was analyzed by the method described in 9 CFR 113.27(d). The estimated titer of this reagent was determined empirically using standard plate count analysis.
9. **Container Size, Type, Weight, or Volume:** 1.0 mL frozen culture
10. **Storage Conditions:** -60°C or colder
11. **CVB Technical Contact:** Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673.
12. **Origin and Passage History:** The organism was first isolated in 1995 from keratin cell culture. The CVB acquired the strain from the EDQM in 2012.

**13. Method of Preparation:** The culture was diluted to a stock concentration recommended by the European Pharmacopoeia in Reference Standard Leaflet Y0000693 in mycoplasma medium supplemented with 15% glycerol. The diluted culture is stored at less than -60°C.

**14. Other:** The estimated titer of this reagent is 32,337 CFU/mL. To achieve a use-dilution containing a target concentration of approximately 100 CFU/mL, prepare a 1:320 dilution in sterile saline. Begin by making a 1:3.2 dilution (0.5 mL culture + 1.1 mL saline), followed by two 10-fold dilutions. Subsequent 10-fold dilutions may be generated to produce 10 CFU/mL, 1 CFU/mL, and 0.1 CFU/mL solutions. To verify the concentration of the dilutions, plate 0.1 mL of each dilution on mycoplasma agar plates and incubate for 10-14 days in a humidified chamber containing 4-6% CO<sub>2</sub> in air or 4-6% CO<sub>2</sub> in nitrogen.

**Restrictions:** This reagent is intended as a one-time use reference standard for the mycoplasma contamination test. Culturing this organism with the intent to propagate a stock culture is not permitted.

Reagent orders and feedback should be sent *including phone number* to the following email address: [CVB@aphis.usda.gov](mailto:CVB@aphis.usda.gov)

Reagent orders forms (APHIS 2018) are available from:  
[https://www.aphis.usda.gov/library/forms/pdf/APHIS\\_2018.pdf](https://www.aphis.usda.gov/library/forms/pdf/APHIS_2018.pdf)

**REVISED:** 18Apr14 alb