1. **Reagent Name:** *Mycoplasma orale*

2. **Strain or Source:** BRP batch 1; European Directorate for the Quality of Medicines & HealthCare (EDQM); Strasbourg, France

3. **Lot Number:** 18Apr12

4. **Fill Date:** April 18, 2012

5. **Expiration Date:** None

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**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 strain was first isolated in 2000 from Vero 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 Y0000691 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 1,962 CFU/mL. To achieve a use-dilution containing a target concentration of approximately 100 CFU/mL, prepare a 1:19 dilution in sterile saline. Begin by making a 1:1.9 dilution by adding 0.9 mL saline to thawed culture and mix gently with a pipette, followed with a 10-fold dilution. 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% CO₂ in air or 4-6% CO₂ 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


**REVISED:** 18Apr14 alb