

**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:** *Leptospira pomona* Challenge Culture
2. **Strain or Source:** MLS, NVSL No. 11000
3. **Lot Number:** IRP 654
4. **Fill Date:** February 23, 2017
5. **Expiration Date:** July 31, 2029

Precautions: *Leptospira* spp. are classified as biosafety level 2 pathogens. Handle under biological safety hood while wearing gloves. Use face shield when inoculating animals.

6. **Intended Use:** Challenge material for *L. pomona* testing.

7. **Instructions for Use:** Remove vials from cold storage and thaw at room temperature (18°C- 27°C) for approximately 1 hour. A water bath or other warming device is not recommended. Do not vortex during thawing. An 18-gauge needle is recommended to remove cryopreserved challenge from the vial. You may draw the contents of more than one vial into a single syringe for convenience. However, to limit spirochete stress and minimize the human safety risk when working with a zoonotic agent, do not actively mix the contents of separate vials.

Inoculate 50-90 g hamsters, preferably four hamsters total, with cryopreserved challenge using a 23-gauge or smaller bore needle. Each hamster should receive at least 0.25 mL IP. At least 50 percent of the inoculated hamsters should exhibit clinical signs within 10 days post-inoculation, and clinical signs within 4-5 days are expected. Hamster death prior to 3 days should not be attributed to leptospirosis. The *Leptospira* must be serially transferred through hamsters a minimum of three times before used as challenge material in a potency test according to Supplemental Assay Method (SAM) 608.

8. **Test of Reagent:** Identity was confirmed by ELISA, MAT, PFGE, REA and MLST analysis. IRP 654 was verified as a virulent challenge when used in potency tests conducted according to SAM 608. The culture was negative for extraneous bacteria when incubated on blood agar plates at 37°C after three passages in hamsters. No hemadsorbing or cytopathogenic viruses were present based on bovine and hamster cells testing. Fluorescent antibody staining verified the absence of the following: REO 1, Rabies, BVDV1, BVDV2, BPV, BTV, BAV1, BAV5 and BRSV. PCR also verified the absence of BVDV1, BVDV2, PCV1, PCV2, and PRRS.

9. **Container Size, Type, Weight, or Volume:** 2.0 mL cryovial containing 1.25 mL hamster liver homogenate with viable *L. pomona*.
10. **Storage Conditions:** For long-term storage, challenge should be maintained in liquid nitrogen. Challenge may be stored at -80°C for storage less than 1 week.
11. **CVB Technical Contact:** Bacteriology Section, Center for Veterinary Biologics, (515) 337-6100.
12. **Origin and Passage History:** Origin: Cornell University through a commercial laboratory (1969). This strain is hamster virulent.
13. **Method of Preparation:** Liver tissue from a *L. pomona* infected hamster is homogenized in liquid P80-BA medium with trimethoprin, sulfamethoxazole, and 5-fluorouracil and then frozen overnight at -80°C. Challenge is stored in liquid nitrogen until use or shipment.
14. **Other:** None.

Reagent orders and feedback should be sent *including phone number* to the following email address: VS.DB.CVB.Reagent.Requests@usda.gov

Reagent order forms (APHIS Form 2018) can be found on the CVB website.