**United States Department of Agriculture**

Animal and Plant Health Inspection Service

Veterinary Services

Washington, DC 20250

April 13, 2012

**VETERINARY SERVICES MEMORANDUM NO. 800.114**

**TO:** VS Leadership Team (VSLT)
Directors, Center for Veterinary Biologics
Biologics Licensees, Permittees, and Applicants

**FROM:** John R. Clifford */s/ John R. Clifford*
Deputy Administrator

**SUBJECT:** Alternative Test Procedure for Tuberculin, PPD Bovis, Intradermic

## I. PURPOSE

The purpose of this memorandum is to inform interested parties that the Center for Veterinary Biologics (CVB) is making available an alternate testing protocol to the test procedure codified in title 9, *Code of Federal Regulations* (9 CFR) section113.409(c) for Tuberculin-PPD Bovis, Intradermic. Information on obtaining an exemption to use the modified test protocol is supplied in this memorandum.

## II. BACKGROUND

9 CFR 113.409(c) outlines the test procedure for potency testing serials of Tuberculin, PPD Bovis, Intradermic (PPD). For this test, 43 guinea pigs are needed: 20 sensitized to *M. avium,* 20 sensitized to *M. bovis, and 3* non-sensitized to be used as controls. The sensitinogens are prepared from heat-killed mycobacterial cells. A reference and test serial of PPD are diluted based on protein content and the dilutions are intradermally injected 35 days after the sensitization injection. Each guinea pig receives four injections of diluted PPD (i.e., different protein concentrations).

An alternate testing protocol has demonstrated more reproducibility and ruggedness in our laboratory. Modifications to our test method are based on protocols from the World Organization for Animal Health (OIE) and the Canadian Food Inspection Agency (CFIA).

The alternate testing protocol uses only 15 guinea pigs, eliminates the *M. avium* sensitized guinea pigs, reduces the number of PPD dilutions for the test, and uses 6 injections per guinea pig. The dilutions are also distributed amongst the guinea pigs differently, so a comparison of the unknown and reference are made within the same guinea pig. The skin reactions are evaluated as a percentage of a reference PPD for the test interpretation.

III. POLICY

Either the codified test (9 CFR 113.409 and SAM 636) or the modified test procedure (BBPRO0002.03 (copy attached)) may be used to test Tuberculin, PPD Bovis, Intradermic. The Outline of Production (the Outline) should specify the protocol that will be used. The CVB laboratory will use the procedure referenced in the Outline when conducting confirmatory testing of serials.

IV. ACTION

In order to obtain an exemption to cite the modified protocol in the Outline, a firm should test three serials with the method codified in 9 CFR 113.409 (further details supplied in SAM 636) and the attached protocol.

These data should be submitted to your reviewer with the necessary changes to the Outline. A Special Outline may be drafted based on the attached protocol. Please ensure the data includes the lots of sensitinogen and reference PPD used for the evaluation. Authorization to submit samples of the three serials for confirmatory testing will then be provided. When satisfactory confirmatory testing is conducted, the alternative protocol can be approved for the firm.

Attachment

# United States Department of Agriculture
# Center for Veterinary Biologics

## Testing Protocol

## Modified Potency Testing Protocol for Purified Protein Derivative (PPD) Tuberculins

Date: **June 29, 2011**

Number: BBPRO0002.03

Supersedes: BBPRO0002.02, June 2, 2011

Contact: Janet M. Wilson, (515) 337-7245
Renee M. Olsen, (515) 337-7467

Approvals:

/s/Geetha B. Srinivas Date: 08Jul11
Geetha B. Srinivas, Section Leader
Bacteriology

/s/Rebecca L.W. Hyde Date: 08Jul11
Rebecca L.W. Hyde, Section Leader
Quality Management
Center for Veterinary Biologics

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

**INTERNAL USE ONLY**

**Modified Potency Testing Protocol for Purified Protein Derivative (PPD) Tuberculins**

## Table of Contents

**Modified Potency Testing Protocol for Purified Protein Derivative (PPD) Tuberculins**

## 1. Introduction

This Testing Protocol (PRO) describes an alternative potency test for evaluation of production lots of tuberculin purified protein derivative (PPD).

Sensitizing inoculums are heat-killed mycobacterial cells suspended in a mixture of mineral oil at a concentration of 20 mg/mL (weight/volume). Guinea pigs are injected intramuscularly (IM) with this inoculum in order to stimulate an immune response to the tuberculo-proteins which are subsequently administered intradermally (ID) when the potency of tuberculin lots are assayed.

## 2. Materials

### 2.1 Instrumentation/equipment

Equivalent instrumentation or equipment may be substituted for any brand name listed below.

**2.1.1** Calibrated digital calipers or metric ruler made of clear plastic

**2.1.2** Needles, 20-gauge x 1-inch and 26-gauge x 3/8-inch

**2.1.3** Disposable Luer-locking syringes, 1-mL and 3-mL

**2.1.4** Pipettes, 1-mL, 2-mL, 5-mL and 10-mL

**2.1.5** Polystyrene tubes to dilute the tuberculin serial to 1.0 mg/mL (if needed)

**2.1.6** Glass serum bottles, 10-mL and 20-mL

**2.1.7** Rubber stoppers for serum bottles

**2.1.8** Aluminum seals for serum bottles

**2.1.9** Crimper for applying aluminum seals

**2.1.10** Animal clippers, equipped with a sharpened #40 or #50 blade

**2.1.11** Ear tags for small animals

**2.1.12** Ear tag applicator

**2.1.13** Cage cards

**2.1.14** Calibrated scale suitable for weighing guinea pigs

**2.1.15** Vivarium specific footwear for animal work

**2.1.16** Scrub pants/top for animal work

**2.1.17** Tape, various colors for identifying each sample

**2.1.18** Personal protective equipment (gloves, eye protection, and/or respirator, if allergies present)

**2.1.19** Hotplate

## 2.2 Reagents/supplies

Equivalent reagents or supplies may be substituted for any brand name listed below.

**2.2.1** International Standard Reference PPD tuberculin, current lot. This reference is obtained from the National Institute for Biological Standards and Control (NIBSC).

**2.2.2** *Mycobacterium bovis* sensitizing agent, current lot, for evaluating *M. bovis* tuberculin serials. This reagent is available from the National Veterinary Services Laboratories (NVSL).

**2.2.3** *Mycobacterium avium* sensitizing agent, current lot, for evaluating *M. avium* tuberculin serials. This reagent is available from the NVSL.

**2.2.4** Sterile Saline, 0.85% – National Centers for Animal Health (NCAH) Media #30201

**2.2.5** Sterile Mineral Oil

**2.2.6** Phosphate Buffered Saline – NCAH Media #10559 with 0.0005% Tween 80

**2.2.7** White, non-pregnant female guinea pigs each weighing 500-700 grams. Twelve guinea pigs are required for each test serial evaluated and 3 additional guinea pigs per serial are held as controls. All guinea pigs must be from same source and housed in the same manner.

## 3. Preparation for the Test

### 3.1 Personnel qualification/training

Technical personnel must have working knowledge of the use of general laboratory chemicals, equipment, and glassware and have specific training and experience in the safe handling of laboratory animals. They must have experience in the performance of this assay.

### 3.2 Selection and handling of test animals

**3.2.1** Select guinea pigs that are healthy and free of external parasites and have an unblemished hair coat.

**3.2.2** Animal care staff will examine the guinea pigs the day they are received, and house them according to the current standard operating procedures.

**3.2.3** At the conclusion of the test, the guinea pigs are euthanized unless they can be used for other purposes.

### 3.3 Preparation of supplies

**3.3.1** All glassware must be sterilized prior to use.

**3.3.2** Only sterile supplies must be used (syringes, needles, rubber seals, etc.).

### 3.4 Preparation of reagents

**3.4.1** Phosphate Buffered Saline - NCAH Media #10559 with 0.0005% Tween 80

| | |
|---|---|
| Sodium chloride | 8 g |
| Potassium chloride | 0.2 g |
| Sodium phosphate dibasic | 1.15 g |
| Potassium phosphate monobasic | 0.2 g |
| Distilled water | QS to 1000 mL |

Autoclave at ≥ 121°C for 20 minutes. When cooled, pH to 7.2 and add 0.5 mL of 1% solution of Tween 80.

1% Tween 80

| | |
|---|---|
| Tween 80 | 0.1 mL |
| Distilled water | QS to 10 mL |

**3.4.2** 0.85% Sterile Saline - NCAH Media #30201

| | |
|---|---|
| Sodium chloride | 8.5 g |
| Distilled water | QS 1000 mL |

Combine and mix well. Autoclave at ≥ 121°C for 20 minutes.

## 4. Performance of the Test

The procedure described below is to evaluate one test tuberculin, that is, to compare one test tuberculin to the appropriate reference standard. Record all potency test information on the current versions of **BBTWS0215** and **BBTWS0210**.

### 4.1 Sensitization

**4.1.1** Heat and maintain the sensitizing inoculum (2 mg [wet weight] heat-killed mycobacterial cells [appropriate species] in 0.1 mL sterile mineral oil) to approximately 45°C by placing the vial in a beaker of water on a hot plate.

**4.1.2** Withdraw the warmed sensitinogen out of the bottle using a 16-gauge needle, then switch to a 20-gauge, 1-inch needle for sensitinogen inoculation. Inject 12 guinea pigs (500-700 g) intramuscularly in the hind leg with 0.1 mL inoculum.

**4.1.3** Allocate 3 unsensitized animals to a control group. Inject these guinea pigs with 0.1 mL sterile saline (also warmed to 45°C).

### 4.2 Guinea Pig Preparation

**4.2.1** Thirty-five ± 2 days after sensitization, clip the hair from the abdomen and sides of each guinea pig as closely as possible to the skin.

**4.2.2** Allow the guinea pigs to rest in their cages a minimum of 4 hours prior to administering tuberculin injections.

### 4.3 Intradermal (ID) Inoculation

**4.3.1** Dilute the test and standard reference tuberculins in PBS with 0.0005% Tween 80 to contain 2, 10, and 50 µg protein/mL (see **BBTWS0209**).

**4.3.2** Label the bottles containing the various dilutions using different colored tape for each sample.

**4.3.3** Insert ear tags in each guinea pig for identification purposes.

**4.3.4** Restrain the guinea pig on its side. First, inject 0.1 mL intradermally of each standard tuberculin dilution (4, 5, and 6) into sites A, B, and C on the left side of the guinea pig. Then position and restrain the guinea pig on its other side to inject 0.1 mL of each test serial dilution (1, 2, and 3) into sites D, E, and F on the right side of the guinea pig. The three dilutions are systematically assigned to the injection sites (see **BBTWS0210**).

**4.3.5** Similarly, inject the three dilutions of the standard and the three dilutions of the serial intradermally on three unsensitized control guinea pigs.

### 4.4 Reading of the skin test

Measure the reactions with calipers 48± 2 hours postinjection. Take two perpendicular diameter readings of the area of erythema of each injection site. Record the measurements on **BBTWS0210**.

## 5. Interpretation of the Test Results

**5.1** The worksheets for data analysis are available upon request.

**5.2** Enter the measurements that have been recorded on **BBTWS0210** into **BBTWS0230**.

**5.3** Calculate the average reaction area of each dilution for both the test tuberculin and reference standard. Calculate the total area of reaction for each tuberculin. The total area of the test tuberculin should be 80-120% of that which is determined for the standard.

## 6. Report of Test Results

Report results of the test(s) as described by standard operating procedures.

## 7. References

CFIA-OLF Standard Operating Procedure SOP #MY-PR041.01

## 8. Summary of Revisions

**Version .03**

**4.3.4:** This section has been revised for clarification.

**Modified Potency Testing Protocol for Purified Protein Derivative (PPD) Tuberculins**

## Version .02

The title of the document has been updated to reflect more accurate verbiage.

**2.1.4:** Pipettes sizes have been updated to reflect equipment that is used in preparation of dilutions.

**2.1.15:** Vivarium specific footwear has been added to reflect the current clothing policy to perform animal work.

**3.2.2:** This section has been updated to reflect procedures performed by the Animal Resources Unit.