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**United States Department of Agriculture
Center for Veterinary Biologics**

Testing Protocol

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

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Table of Contents

- 1. Introduction**
- 2. Materials**
 - 2.1 Instrumentation/equipment**
 - 2.2 Reagents/supplies**
- 3. Preparation for the Test**
 - 3.1 Personnel qualifications/training**
 - 3.2 Selection and handling of test animals**
 - 3.3 Preparation of supplies**
 - 3.4 Preparation of reagents**
- 4. Performance of the Test**
- 5. Interpretation of the Test Results**
- 6. Report of Test Results**
- 7. References**
- 8. Summary of Revisions**

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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** Sprayola animal paint and applicator
- 2.1.13** Ear tag applicator
- 2.1.14** Cage cards

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2.1.15 Calibrated scale suitable for weighing guinea pigs

2.1.16 Vivarium specific footwear for animal work

2.1.17 Scrub pants/top for animal work

2.1.18 Tape, various colors for identifying each sample

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

2.1.20 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.

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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 $\geq 121^{\circ}\text{C}$ 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

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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 $\geq 121^{\circ}\text{C}$ 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 **CVB-TWS-0049**, *Guinea Pig Potency Test Tuberculin Purified Protein Derivative (PPD) Modified Testing Protocol Worksheet*, and **CVB-TWS-0047**, *Modified PPD Potency Testing Record Worksheet*.

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.

The standard NVSL stock sensitinogen is 100 mg (wet weight) heat-killed mycobacterial cells (appropriate species) per mL. When sensitizing the guinea pigs with standard NVSL stock sensitinogen, a reasonable dilution scheme is 2 mL NVSL stock sensitinogen and 3 mL of sterile mineral oil diluent for a final concentration of 40 mg/mL of sensitizing inoculum.

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 \pm 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.

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4.3 Intradermal (ID) Inoculation

4.3.1 Dilute the test and standard reference tuberculin in PBS with 0.0005% Tween 80 to contain 2, 10, and 50 µg protein/mL (see **CVB-TWS-0047**, *Modified Potency Testing Protocol PPD Dilutions Worksheet*).

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. Alternatively, mark guinea pigs with Sprayola to differentiate them. Animals may be marked with different colors on the head, back, rear, or foot for identification.

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 **CVB-TWS-0049**).

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 post injection. Take two perpendicular diameter readings of the area of erythema of each injection site. Record the measurements on **CVB-TWS-0049**.

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 **CVB-TWS-0049** into **CVB-TWS-0052**, *PPD Tuberculin Summary Worksheet*.

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.

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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 CVB-PRO-0001.03

- Updated to DT format.

Version CVB-PRO-0001.02

- Removed title page and contacts to match Master Control processes.
- Updated alphanumeric identification of this document and cited documents to match current processes.

Version BBPRO0002.06

- **4.1.1:** Updated NVSL sensitinogen concentration based on current practices.

Version BBPRO0002.05

- Updated Section Leader.
- Alternative guinea pig identification method added.

Version BBPRO0002.04

- **4.1.1:** Clarified the dilution of sensitinogen.
- Updated contact person

Version BBPRO0002.03

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

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Version BBPRO0002.02

- 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.