Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

**WHO International Standard**
**Purified Protein Derivative (PPD) of Mycobacterium bovis**
**Tuberculin**
**NIBSC code: PPDBOV**
**Instructions for use**
**(Version 6.0, Dated 14/10/2010)**

## 1. INTENDED USE

The 1st International Standard for PPD of Mycobacterium bovis Tuberculin was donated by Central Diergeneeskundig Instituut, Rotterdam, Netherlands, in 1986. This material has been prepared and characterised by the Veterinary Laboratories Agency (VLA), Weybridge, Surrey, UK. The package insert from VLA is attached. This material is intended for use in the calibration of the contents of 'effective constituent' in national or working standard preparations and for the expression of these contents in the respective International Units.

## 2. CAUTION

**This preparation is not for administration to humans or animals in the human food chain**.

The material is not of human or bovine origin. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

## 3. UNITAGE

Each ampoule contains 58 500 International Units (IU) of PPD.

## 4. CONTENTS

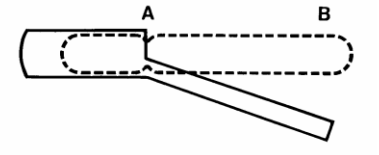
Country of origin of biological material: Netherlands.
Each ampoule was filled with 1.8 mg (standard deviation 0.024%) of purified PPD in glucose phosphate buffer containing phenol. Each ampoule contains 58 500 International Units (IU) of PPD.

## 5. STORAGE

For long-term storage, this material should be stored at -20ºC.
**Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.**

## 6. DIRECTIONS FOR OPENING

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.




Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

## 7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
The entire contents of each ampoule should be completely dissolved in an accurately measured amount of appropriate solvent (distilled water, saline or buffer) or as recommended in the VLA insert.

## 8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

## 9. REFERENCES

## 10. ACKNOWLEDGEMENTS

VLA, WHO

## 11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

## 12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

## 13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

## 14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

| **Physical and Chemical properties** | |
|---|---|
| Physical appearance: Freeze dried solid | Corrosive: No |
| Stable: Yes | Oxidising: No |
| Hygroscopic: No | Irritant: No |
| Flammable: No | Handling: See caution, Section 2 |
| Other (specify): Contains material of mycobacterial origin with glucose, phosphate and phenol | |
| **Toxicological properties** | |
| Effects of inhalation: Not established, avoid inhalation | |
| Effects of ingestion: Not established, avoid ingestion | |

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| |
|---|
| Effects of skin absorption: Not established, avoid contact with skin |
| **Suggested First Aid** |
| Inhalation: Seek medical advice |
| Ingestion: Seek medical advice |
| Contact with eyes: Wash with copious amounts of water. Seek medical advice |
| Contact with skin: Wash thoroughly with water. |
| **Action on Spillage and Method of Disposal** |
| Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water.<br>Absorbent materials used to treat spillage should be treated as biological waste. |

**15. LIABILITY AND LOSS**

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

**16. INFORMATION FOR CUSTOMS USE ONLY**

| |
|---|
| **Country of origin for customs purposes*:** United Kingdom<br>* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying. |
| **Net weight:** 1.8 mg |
| **Toxicity Statement:** Toxicity not assessed |
| **Veterinary certificate or other statement** if applicable**.**<br>**Attached:** No |

**17. CERTIFICATE OF ANALYSIS**

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

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**Central Veterinary Laboratory**
New Haw, Addlestone, Surrey KT15 3NB United Kingdom
Telephone: 01932 357641 Facsimile: 01932 357701 Telex: 262318 VetWey G

Veterinary Laboratories Agency

# INTERNATIONAL STANDARD FOR TUBERCULIN, PURIFIED PROTEIN DERIVATIVE (PPD), BOVINE (PPD BOV)

## Description

The International Standard for Purified Protein Derivative (PPD) of *Mycobacterium bovis* Tuberculin was donated by the Central Diergeneeskundig Instituut, Netherlands.

The Standard was prepared as a single homogenous bulk. Each neutral glass ampoule was filled with 1.8mg (standard deviation 0.024%) of purified protein derivative in a glucose phosphate buffer containing phenol. The material was freeze-dried and sealed under vacuum.

## International Unit

Each ampoule contains 58,000 International Units of PPD and when reconstituted with 1.8ml of diluting fluid will contain 1mg PPD and 32,500 IU per ml.

## Distribution

The Standard is distributed by the International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Addlestone, Surrey, England on behalf of the World Health Organisation. It is available free of charge in limited amounts. If a laboratory needs more than one sample every six months, it is expected to prepare its own standard and to calibrate it against the International Standard. A quantity of this latter sufficient for the purpose will be supplied on request.

## Reconstitution of the International Standard

The Standard should be reconstituted immediately before it is to be used.

Ampoules may be opened by scoring with a small saw specifically designed for the purpose, or a hard mineral edge, for approximately one third of the circumference. Application of a piece of red hot glass rod to this scratch will give a clear line of fracture.

If the scoring is made firmly and the glass rod is hot enough, it is possible to produce a fine crack without disturbing the ampoule top until needed. Then slight pressure will complete the separation.

To the contents of the ampoule add 1.8ml of 0.32% solution of phenol in distilled water. This gives a solution in phosphate buffer containing phenol (0.5%) and glucose.

Care should be taken to ensure that the entire contents of the ampoule are completely resuspended. This can be achieved by suspending the bulk of the contents of the ampoule in some of the fluid and using the remainder of the diluent to rinse out the ampoule three times.

*An Executive Agency of the Ministry of Agriculture, Fisheries and Food*

**National Working Reference Standard**

National and working reference standards should be prepared in a stable form. This may be achieved by freeze-drying aliquots of the reference preparation to neutral-glass ampoules and sealing them in a vacuum by fusion of the glass. The ampoules should be stored in the dark at a low temperature, eg -20°C.

**Calibration of National and Working Reference Standards**

It is recommended that the International Standard is used to calibrate a working reference standard and that the calibration assay be carried out in cattle.

The potency of the working reference standard relative to that of the International Standard is calculated and the results expressed in International Units.

It is suggested that the potency of a working reference standard should be checked against that of a fresh sample of the International Standard about once a year.

The International Laboratory for Biological Standards at Weybridge is willing to advise and assist laboratories in providing national and laboratory standards.

**Diluting Fluid for Assays**

The diluent consists of isotonic phosphate-buffered saline pH 7.3, containing Tween 80, (0.0005%), and is prepared by adding 0.5ml of 1% w/v solution of Tween 80 in distilled water to 1 litre of the following solution:-

| | |
|---|---|
| $Na_2HPO_42H_4O$ | 7.60g |
| $KH_2PO_4$ | 1.45g |
| NaCl | 4.80g |
| Distilled water to | 1 litre |

Tuberculin undiluted and diluted 1 in 5 are usually satisfactory for cattle assays while dilutions of 1 in 100, 1 in 500 and 1 in 2500 with a 0.1ml inoculum or 1 in 200, 1 in 1000 and 1 in 5000 with a 0.2ml inoculum generally will produce satisfactory results in guinea-pig assays.

**Assays in Cattle**

a) Assay Design

Inject the tuberculins in a dose of 0.1ml into the neck, the dilutions being randomly allocated to the 3 sites on each side of the neck. Measure the skin thickness at each site at the time of injection and after 72 hours.

b) Relative Potency

Perform assays in at least 30 cattle. If necessary, select groups of 6 animals with a similar history of breeding, age and sex. The animals could be naturally sensitised, as confirmed by a recent test with bovine tuberculin. Alternatively, calves could be artificially infected with a living culture of *M. bovis*. The calves should be more than six months old at the time of infection and the first test should be performed six to eight weeks later.



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**Assays in Guinea-pigs**

**Sensitisation of guinea-pigs**

Select a group of at least 9 albino guinea-pigs each weighing not less than 400g. Inject each animal intramuscularly on the medial side of the thigh with 0.0001mg wet weight *M. bovis*, living strain AN5, suspended in 0.5ml physiological saline.

**Assay design**

Carry out each assay in the group of 9 guinea-pigs, between 4 and 6 weeks after sensitisation. Test three dilutions of each of 3 preparations. Since it is practicable to give only 8 injections to an individual animal, a balanced incomplete Latin Square design is used, in which a different one of the 9 dilutions is omitted from each animal. The remaining 8 dilutions are allocated to 4 sites.



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