

**College ter Beoordeling van Geneesmiddelen (CBG)
Medicines Evaluation Board (MEB)**

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**DECENTRALISED
PROCEDURE**

**PUBLICLY AVAILABLE ASSESSMENT REPORT FOR A VETERINARY
MEDICINAL PRODUCT**

Tuberculin PPD Kit

March 2019

MODULE 1

PRODUCT SUMMARY

EU Procedure number	NL/V/0322/001
Name, strength and pharmaceutical form	Tuberculin PPD Kit
Applicant	Prionics Lelystad B.V
Active substance(s)	Bovine Tuberculin PPD, Avian Tuberculin PPD
ATC Vetcode	Q102AR01
Target species	Cattle
Indication for use	To be used for the <i>in-vivo</i> identification of cattle which have been exposed to <i>Mycobacterium</i> species causing bovine tuberculosis. Simultaneous administration of Bovine Tuberculin PPD 3000 and Avian Tuberculin PPD 2500 and comparing the degree of skin swelling of the two injection sites enables differentiation between the specific reaction in target species exposed to <i>Mycobacterium</i> species causing bovine tuberculosis and the non-specific reaction in target species, which have been exposed to other species of <i>Mycobacteria</i> or related genera, causing cross sensitisation to Bovine Tuberculin PPD 3000.

MODULE 2

The Summary of Product Characteristics (SPC) for this product is available on the following website:

<https://www.diergeneesmiddeleninformatiebank.nl/en/>

MODULE 3

PUBLIC ASSESSMENT REPORT

Legal basis of original application	MUMS (Minor Use Minor Species) DCP application in accordance with the EMA Guideline on Data Requirements for Immunological Veterinary Medicinal Products intended for Minor use or Minor species/ limited markets, (EMA/CVMP/IWP/123243/2006-Rev.2).
Date of completion of the original decentralised procedure	25 th August 2010.
Date product first authorised in the Reference Member State (MRP only)	Not applicable.
Concerned Member States for original procedure	Ireland, The Netherlands, UK (RMS).

I. SCIENTIFIC OVERVIEW

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis* is a cause of major production loss throughout the world. The detection of infected animals and exposed cattle permits culling and thus control and eradication. *In vivo*, diagnostic tests based on intradermal application and subsequent skin reaction to purified protein derivatives (PPDs) have been effectively used for many decades. To date, no reliable vaccines or blood tests have been developed. *Mycobacterium avium* as well as other mycobacterial species can cause an immune response or sensitisation, with no associated pathology in the target species, (cattle). The Tuberculin PPD Kit contains two diagnostic reagents, bovine tuberculin PPD and avian tuberculin PPD, derived from *M. bovis* and *M. avium* respectively, which are used via intradermal injection, to cause a comparable immune response. Quantitative comparison between the size of the two skin reactions induced by both reagents, allows for the identification of cattle exposed to *M. bovis* or other *Mycobacterium* species causing bovine TB.

The pharmaceutical form of the product is a solution for injection. The simultaneous administration of two injections, one containing bovine tuberculin PPD and one containing avian tuberculin PPD, provides a platform for the comparison of reactions caused by a specific immune response to *Mycobacterium* species causing tuberculosis, as compared to a non-specific reaction related to a non-pathogenic *Mycobacterium* species. The product contains purified protein derivatives (PPDs) derived from cultures of *Mycobacterium bovis* strain AN5, and *Mycobacterium avium* strain D4ER.

The Summary of Product Characteristics (SPC) provides further details with regard to use of the product, with relevant warnings. The product is produced and controlled using validated methods and tests which ensure the consistency of the product released on the market. It has been shown that the product can be safely used in the target species, the reactions observed are indicated in the SPC. The product is safe for the user, the consumer of foodstuffs from treated animals and for the environment, when used as recommended. The efficacy of the product was demonstrated according to the claims made in the SPC. The overall benefit/risk analysis is in favour of granting a marketing authorisation.

This application was made under the European Guidelines for MUMs applications. A MUMS Marketing Authorisation (Minor Use, Minor Species), is intended for use where limited numbers of a product are expected to be sold, and where a need for a specific drug is required. Due to the nature of the use of such drugs, a full dossier meeting all requirements for the marketing of the drug is not required, provided that the safety, quality and efficacy of the product can be supported by available data, and a favourable benefit-risk profile is determined. There is a requirement for Periodic Safety Update Reports (PSURs) to be submitted for annual reassessment of this product. Moreover, results of any additional studies must be submitted to the Veterinary Medicines Directorate (VMD).

II. QUALITY ASPECTS

A. *Composition*

Tuberculin PPD Kit is composed of two components: Bovine Tuberculin PPD 3000 and Avian Tuberculin PPD 2500. Bovine Tuberculin PPD 3000 contains tuberculin PPD derived from cultures of the AN5 strain of *M. bovis* and has a potency of 3000 IU¹ per 0.1 ml dose. Avian Tuberculin PPD 2500 contains tuberculin PPD derived from cultures of the D4ER strain of *M. avium* and has a potency of 2500 IU per 0.1 ml dose. The excipients for both components are phenol, anhydrous glucose, disodium phosphate dihydrate, potassium dihydrogen phosphate and water for injections. Additionally, Avian Tuberculin PPD 2500 contains Ponceau 4R (E 124) to make it easier for the user to differentiate between the two components of the Tuberculin PPD Kit.

The container system consists of two presentations². The first presentation is a polystyrene box containing 20 (hydrolytic Type 1) vials (hydrolytic Type I), of Bovine Tuberculin PPD 3000 and 20 vials of Avian Tuberculin PPD 2500. Each vial contains 20 test doses of 0.1 ml. The second presentation is a polystyrene box containing 10 vials (hydrolytic Type 1) of Bovine Tuberculin PPD 3000 and 10 vials of Avian Tuberculin PPD 2500. Each vial contains 50 test doses of 0.1 ml. The particulars of the containers and controls performed are provided and conform to the regulation.

¹ IU = International Units.

² As a result of a variation procedure, an additional presentation comprising 50 doses was introduced. The variation also amended the transport temperature for the product. (See Section G).

The choice of *Mycobacterium* strains, inactivating process and the presence of preservative are justified. The inactivation process and the detection limit of the control of inactivation are correctly validated. The product is an established pharmaceutical form and its development is adequately described in accordance with the relevant European guidelines.

B. Method of Preparation of the Product

The product is manufactured fully in accordance with the principles of good manufacturing practice (GMP) from a licensed manufacturing site. Process validation data on the product have been presented in accordance with the relevant European guidelines.

The basic manufacturing process continues to be predominantly that designed in the 1950s, but developments have occurred due to GMP and European Pharmacopoeial (Ph. Eur) requirements. Essentially, the manufacturing process consists of the growth and heat inactivation of Mycobacterial cultures, followed by clarification, purification, concentration, stabilisation and blending of the tuberculin purified protein. The blended tuberculin PPD is formulated to a bulk. The bulk is sterile filtered prior to filling, and the filling process is followed by assembly and packaging of the final product.

C. Control of Starting Materials

The active substances are established active substances and are manufactured in accordance with the principles of GMP. There are monographs for both bovine and avian tuberculin PPDs in the Ph.Eur. The *M. bovis* and *M. avian* Master Seeds originated from seed materials held at the Animal and Plant Health Agency, Weybridge, UK.

The identity and purity of both Master Seeds was verified according to specific Ph. Eur monographs. Other materials of a biological origin, not mentioned in a pharmacopoeia are tryptone, beef extract, egg and potato.

Starting materials of non-biological origin used in production comply with the Ph. Eur where appropriate. Biological starting materials used are in compliance with the relevant Ph. Eur. monographs and guidelines, and are appropriately screened for the absence of extraneous agents, any deviation was adequately justified.

The master and working seeds have been produced according to the Seed Lot System as described in the relevant guideline.

D. Specific Measures concerning the Prevention of the Transmission of Animal Spongiform Encephalopathies

Scientific data and certificates of suitability issued by the EDQM have been provided and compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been satisfactorily demonstrated.

E. Control tests during production

The tests performed during production are described and the results of 3 consecutive runs, conforming to the specifications, are provided. Tests include a visual inspection of seeding and production cultures and related media, a test for absence of viable mycobacteria following inactivation, determination of pH during purification and concentration, sterility, cell count, fill volume and visual appearance of the vials of the two components of the finished product (Tuberculin PD Kit).

F. Control Tests on the Finished Product

The tests performed on the two components of the finished product conform to the relevant requirements, and deviation from these requirements is justified. The tests include in particular analysis of potency and identity of both active substances, determination of pH, sterility, toxicity, phenol content, osmolarity, protein content, sensitising effect, visual inspection of the Tuberculin PPD Kit and the matching of potencies for the two components of the Tuberculin PPD Kit.

The demonstration of the batch to batch consistency is based on the results of 3 batches produced according to the method described in the dossier. Other supportive data provided confirm the consistency of the production process.

G. Stability

Stability data on the active substances have been provided in accordance with applicable European guidelines, demonstrating the stability of the active substance when stored under the approved conditions.

Data were presented on the stability of concentrated harvests of the product (the active substance) up to 36 months. Potency tests performed with pilot batches formulated with active substance of 3, 27 and 39 months old showed that no deterioration of the product occurred over these time frames. Therefore concentrated harvests of bovine and avian tuberculin PPD may be stored at 2°C-8°C for 3 years.

A series of studies were performed on the bovine and avian tuberculin PPD final products, (20 dose presentation) over 24 hours, 2 weeks and 39 months. Storage was at approximately 25°C for the 24 hours and at approximately 25°C and 37°C for the 2 week period, and approximately 5°C for the 39 month period.

The in-use shelf-life of the broached product that was proposed could not be supported by the data provided. Therefore the product should be used immediately. The product may be transported at +2°C - +37°C for a period of no longer than 14 days.

H. Genetically Modified Organisms

Not applicable.

J. Other Information

The Tuberculin PPD Kit comprises two components each with a different shelf-life.

Shelf-life of the 20-doses presentation:

Bovine Tuberculin PPD 3000: 4 years

Avian Tuberculin PPD 2500: 4 years

Shelf-life of the 50-doses presentation:

Bovine Tuberculin PPD 3000: 4 years

Avian Tuberculin PPD 2500: 4 years

The shelf-life of the Tuberculin PPD Kit is specified as the shelf-life of the component with the earliest expiry date.

Shelf-life after first opening of the container: the product should be used immediately after first opening.

III. SAFETY ASSESSMENT

Diagnostic tests based on the intradermal application of PPDs and the analysis of ensuing skin reactions have been used for many decades. A delayed hypersensitivity reaction is formed in animals where previous exposure to a specific pathogen has occurred. The tuberculin PPD test may be performed in two ways; in the first instance a single injection is used to generate an absolute skin reaction, which determines the result. In the second instance, a single intradermal comparative cervical tuberculin (SICCT) test consists of the administration of both bovine and avian tuberculin, whereby a comparative skin test determines the result. The Tuberculin PPD Kit is specifically to be used in the SICCT test. Refer to the SPC for further information on the test procedure and the interpretation of results.

There are no stipulations with regard to safety testing in the Ph. Eur monographs for bovine and avian tuberculin PPDs. Both tuberculins are categorised under 'Diagnostics' in the Guideline on Data Requirements for Immunological Veterinary Medicinal Products intended for Minor Use or Minor Species/Limited Markets, (MUMS Guideline). Data requirements for this product comply with this ruling, and therefore there was no requirement for the applicant to use maximum potency batches in safety tests. For MUMS applications, literature references may be used to support safety and efficacy claims.

Laboratory trials

Two studies were presented in support of the administration of one dose, an overdose and the repeated administration of one dose. The tests combined the application of one overdose of each tuberculin, followed by a single dose.

In the first study, the safety of the intradermal administration of an overdose was followed 2 weeks later by a normal dose of the components of the Tuberculin PPD Kit, to young, conventional (not previously sensitised), cattle. The study

was conducted in line with good laboratory practise (GLP) requirements. Cattle were assigned to groups, and given a double dose of tuberculin on the right side of the neck (0.2ml of each PPD), in the first instance, followed by a single dose of tuberculin on the left side of the neck, (0.1 ml of each PPD) 14 days later. A control group received only saline as an injection. The animals were monitored over the period of the study, and skin thickness measured. At necropsy, injection sites were examined macroscopically and microscopically. No adverse reactions attributable to the tuberculin were observed.

A second study analysed the safety of an SICCT test in cattle naturally infected with *Mycobacterium bovis*. A suitable number of young bTB cattle were assigned to two groups. One group served as a control group. The test group received a double dose of tuberculin on the right side of the neck (0.2 ml of each PPD) in the first instance, followed by a single dose on the left side of the neck (0.1 ml of each PPD) 14 days later. Cattle were monitored for 2 weeks after each injection, with local reactions and physiological parameters being examined. At necropsy, injection sites were examined macroscopically and microscopically. No adverse reactions attributable to the tuberculin were observed.

The product is inactivated and thus the specific tests to be performed for live vaccines are not applicable.

Supporting data were provided in the form of literature references. The first report was related to the regressing tuberculin skin reaction in cattle. A large number of SICCT positive cattle originating from herds with confirmed bovine tuberculosis were divided into groups. Another group of SICCT negative cattle from a bovine tuberculosis-free herd had been grazing on pasture which had previously led to avian tuberculin reactions. All animals were given a long period of time between SICCT test and study injections. Results confirmed that the maximum size of the injection site in reactive animals was reached at 72 hours post-injection. A rapid regression followed within the first week, followed by a slower resolution.

A further publication was presented which described a sequential study of the bovine tuberculin reaction, identifying leucocyte populations involved in the immune response. A group of SICCT-positive animals, originating from infected herds was inoculated using the SICCT test. There were at least 43 days between tests. Maximum thickness in positive reactors, (all animals), was exhibited at 72 hours post-injection. Gamma delta T-cells were the most numerous leucocyte found in the perivascular fluid post-injection.

No specific laboratory studies were performed with regard to use of the product during pregnancy or lactation. The SPC cites an indication that field experience of the administration of bovine and or avian tuberculosis does not have a negative effect on reproduction or lactation.

Neither of the components of the product are likely to have a detrimental effect on immune function. Repeated testing leads to desensitisation, thus the recommendation in the SPC is to leave at least 42 days between tests, in order to avoid diminution of the visible immune response.

Residue testing was not applicable, as the product contains no materials which require a Maximum Residues Limit.

It is recommended that no other products be used near the injection site of the Tuberculin PPD Kit components during the period immediately before vaccination, and until the TB-specific reactions are interpreted. The SPC also highlights other instances in which the Tuberculin PPD Kit, or other veterinary medicinal products should not be used.

Field studies

Literature references were submitted in the absence of a Good Clinical Practice trial. Both publications related to the bovine tuberculosis eradication programme carried out in Ireland. It was noted that no adverse effects attributable to the Tuberculin PPD Kit were seen, highlighting the safe use of Tuberculin PPD Kit in a large numbers of animals, including all ages, all trimesters of pregnancy as well as administration during lactation.

Ecotoxicity

The applicant provided a Phase I environmental risk assessment in compliance with the relevant guideline which showed that no further assessment was required. The assessment concluded that because the PPDs are inactivated, and all other constituents are present in small amounts, most of the product will be metabolised and eliminated. The product will be delivered by a professional from vials containing small quantities, and the warnings and precautions as listed on the product literature are adequate to ensure safety to the environment when the product is used as directed. The SPC describes precautions to be used by the user, which consist of the avoidance of self-injection, the occurrence of any immune reaction, and the recommended wearing of personal protective clothing. If a strong reaction or systemic symptoms occur, the advice of a physician must be sought immediately.

IV CLINICAL ASSESSMENT (EFFICACY)

Clinical Studies

Laboratory Trials

The efficacy of the product has been demonstrated in a laboratory study in accordance with the relevant requirements. In this study, the efficacy of the Tuberculin PPD Kit was tested in cattle experimentally infected with *M. bovis* or *M. avium*. Three groups of cattle were screened using the SICCT test, in order to demonstrate that they were free of previous exposure to Mycobacterial spp. After the validation testing, animals were re-tested by SICCT just before infection. For all tests, only animals presenting with a suitable reaction were recruited for the study. The cattle were placed in either a test group or control group, and further tested some weeks after infection. Blood samples were collected at various time-points, in order to confirm successful infection. Cattle infected with *M. bovis* and *M. avium* displayed raised levels of gamma interferon over time, with all animals in each group shown to be infected. Controls remained negative. A standard interpretation of ensuing skin reactions demonstrated the suitability of the Tuberculin PPD Kit components as being sufficiently sensitive and specific for routine use.

Field Trials

The applicant provided bibliographical data consisting of a series of publications. A reference was provided which gave background information in relation to the use of tuberculin preparations in Ireland since 1960, up until 2004. A second report analysed data from the UK National surveillance database, Vet Net, comparing the performance of tuberculins produced by APHA Weybridge and by Lelystad (now Prionics Lelystad B.V.) over a period of 2 years. Any cattle testing positive by the SICCT test were slaughtered. Post-mortem results were recorded as part of the National programme. For both tuberculins, the following parameters were examined: skin reaction, probability of confirmed *M. bovis* infection in reactors, inclusive reactors and direct contacts following slaughter, probability of confirmed *M. bovis* culture in reactors, rate of reactors and inconclusive reactors per 100,000 animals, and incidence of herd breakdown. Results indicated that approximately 35% of animals testing skin-positive will be confirmed as having disease via post-mortem or culture. The Lelystad Tuberculin PPD Kit is highly comparable to the tuberculin previously produced by APHA Weybridge.

A further reference analysed the performance of the single intradermal comparative tuberculin test in identifying cattle with tuberculous lesions. A suitable number of cattle identified for slaughter due to exposure to bovine tuberculosis were given the test prior to being sacrificed. Positive and inconclusive reactors were slaughtered approximately one week after testing, negative animals were slaughtered two to three weeks after testing. The test was performed using Prionics Lelystad bovine tuberculin PPD 3000 and avian tuberculin 2500, according to the SPC. Only animals with visible lesions were studied further, as others may have been infected post-test. The overall sensitivity of the SICCT test was approximately 91%, with specificity measured at 78.8%, as measured against autopsy. The usefulness of the kit in the field was therefore demonstrated.

V OVERALL CONCLUSION AND BENEFIT– RISK ASSESSMENT

The data submitted in the dossier demonstrate that when the product is used in accordance with the Summary of Product Characteristics, the benefit/risk profile for the target species is favourable and the quality and safety of the product for humans and the environment is acceptable.

MODULE 4

POST-AUTHORISATION ASSESSMENTS

The SPC and package leaflet may be updated to include new information on the quality, safety and efficacy of the veterinary medicinal product. The current SPC for this product is available on the Product Information Database of the Medicines Evaluation Board (MEB):

<https://www.diergeenesmiddeleninformatiebank.nl/en/>

This section contains information on significant changes which have been made after the original procedure which are important for the quality, safety or efficacy of the product.

•	18 March 2021	Repeat-Use
•	25 April 2019	Change the procedure number from UK/V/0342/001 to NL/V/0322/001
•	05 April 2018	Change in the specification parameters of the finished product. Correction of the quantitative composition of 3 components. Correction of fill volume units. Change in unit in the header of the tables for blending. Corrections and inclusion of holding times of intermediate product. To update name of supplier. Changes in the manufacturing process of the active substance.
•	14 June 2017	Increase in the shelf-life of the finished product, from 3 years to 4 years
•	16 August 2016	Replacement of the current Seed Lot Systems for <i>M. bovis</i> and <i>M. avium</i> with a new Seed Lot System for each Mycobacterium. Change in the manufacturing process of the active substance. Change in the manufacturing process of the active substance.
•	13 April 2016	Increase in shelf life for Avian Tuberculin PPD 2500, 20-doses presentation from 3 years to 4 years.
•	17 November 2015	Increase in the shelf-life of the finished product as packaged for sale.
•	07 May 2015	Renewal – UK as RMS.
•	17 July 2014	Deletion of non-significant in-process controls. Addition of a secondary supplier for a component used in the manufacturing process

		of the active substance.
•	17 April 2014	Extension of shelf life from 24 to 30 months for the Bovine Tuberculin PPD component, addition of a supplier of the packaging components and change of name of the manufacturer of packaging components.
•	8 November 2013	Change in specification parameters and test procedures for the finished product.
•	26 April 2013	Variation to extend the shelf life of the two components of the Tuberculin PPD kit for the 50 dose presentation from 18 months to 2 years.
•	02 November 2012	Variation to extend the shelf-life of the two components of Tuberculin PPD for the 50-dose presentation: from 12 to 18 months.
•	22 March 2012	Text changes to SPC and package leaflet.
•	24 January 2012	Changes in the manufacturing process of the active substance.
•	12 January 2012	Changes to an existing pharmacovigilance system as described in the DDPS.
•	12 January 2012	Changes to an existing pharmacovigilance system as described in the DDPS.
•	7 December 2010	Addition of pack size of the finished product and amendment of transport temperature to +2°C – 37°C for a period of no longer than 14 days.