PROVA GAMMA INTERFERON E SOP 004/EURL

Ivonne Archetti
Centro di Referenza Nazionale per la Tubercolosi Bovina
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Workshop CRN-TB-21 Giugno 2022

DIAGNOSIS OF TUBERCULOSIS INFECTION IN BOVINE AND CAPRINE ANIMALS FOR USING THE IN VITRO GAMMA-INTERFERON DETECTION ASSAY.

APPLIED BIOSYSTEMS™ BOVIGAM™ TB KIT (THERMO FISHER SCIENTIFIC)

SOP/004/EURL

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DIAGNOSIS OF TUBERCULOSIS INFECTION IN BOVINE ANIMALS FOR USING THE IN VITRO GAMMA-INTERFERON DETECTION ASSAY.

ID Screen® Ruminant IFN-g (IDvet)

SOP/006/EURL

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DELLA LOMBARDIA E DELL'EMILIA ROMAGNA
"BRUNO UBERTINI"
ENTE SANITARIO DI DIRITTO PUBBLICO

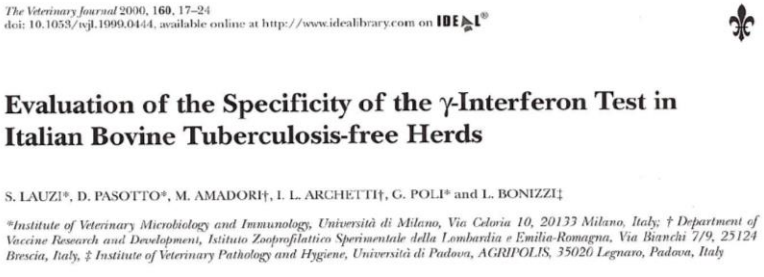
1990

P R Wood 1, L A Corner, P Plackett «Development of a simple, rapid in vitro cellular assay for bovine tuberculosis based on the production of gamma interferon» Res Vet Sci. 1990 Jul;49(1):46-9


LAVORI ORIGINALI

IMPIEGO DEL TEST γ-INTERFERON PER LA DIAGNOSI DI TUBERCOLOSI BOVINA IN REGIONE LOMBARDIA

1996


The Veterinary Journal 2000, 160, 17–24
doi: 10.1053/tvjl.1999.0444, available online at http://www.idealibrary.com on IDEAL®

Evaluation of the Specificity of the γ-Interferon Test in Italian Bovine Tuberculosis-free Herds

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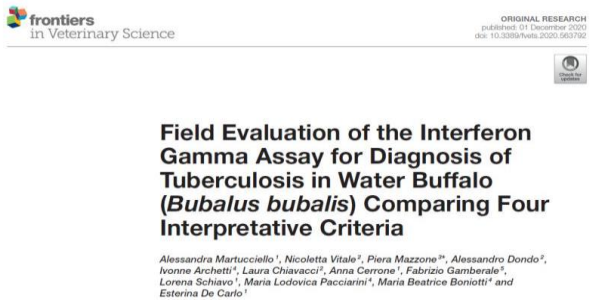
2000


frontiers in Veterinary Science

ORIGINAL RESEARCH
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Field Evaluation of the Interferon Gamma Assay for Diagnosis of Tuberculosis in Water Buffalo (*Bubalus bubalis*) Comparing Four Interpretative Criteria

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2020

Alessandra Martucciello, Nicoletta Vitale, Piera Mazzone, Alessandro Dondo, Ivonne Archetti, Laura Chiavacci, Anna Cerrone, Fabrizio Gamberale, Lorena Schiavo, Maria Pacciarini, Maria Beatrice Boniotti, Esterina De Carlo "Field Evaluation of the Interferon Gamma Assay for Diagnosis of Tuberculosis in Water Buffalo (Bubalus bubalis) Comparing Four Interpretative Criteria". Front. Vet. Sci. 1:563792. doi: 10.3389/fvets.2020563792

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KIT Bovigam

2002

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CSL animal health

BOVIGAM™
Bovine Gamma Interferon Test

ENZYME IMMUNOASSAY (EIA) FOR THE DETECTION OF BOVINE γ INTERFERON

Instructions for use
For kits containing 192 Wells, 960 Wells, or 2880 Wells

for research use only

AN ASSAY OF CELL MEDIATED IMMUNITY FOR USE IN CATTLE, SHEEP, GOATS AND OTHER BOVIDAE

Updated: February 2002

CSL animal health

2022

applied biosystems — INSTRUCTIONS FOR USE

BOVIGAM™ TB Kit

An *in vitro* diagnostic test kit for detection of bovine tuberculosis infection in cattle

Catalog Number 63320, 63326 — Pub. No. MAN0017015 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at thermofisher.com. Wear appropriate protective eyewear, clothing, and gloves.

Introduction

Tuberculosis, a disease caused by *Mycobacterium bovis* infection of cattle, occurs in every country of the world and is of major importance to the dairy cattle industry. In some countries, the overall incidence of disease in individual dairy herds may approach a morbidity rate of 60–70%.

Description

Applied Biosystems™ BOVIGAM™ TB Kit is a rapid in vitro blood-based assay of cell mediated response to *M. bovis* PPD tuberculin for the diagnosis of bovine tuberculosis infection in cattle. Tuberculin PPD antigens are presented to lymphocytes in whole blood culture. The production of IFN-γ from the cells is then detected using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). Lymphocytes from cattle not infected with *M. bovis* do not produce IFN-γ. Therefore, detection of IFN-γ correlates to *M. bovis* infection.

Field studies

Field trials in over 13,000 head of cattle in Australia, USA, Ireland, New Zealand, Italy and Spain have shown that BOVIGAM™ is more sensitive than the intra-dermal tuberculin test for the diagnosis of bovine tuberculosis and may even detect *M. bovis*-infected cattle at an earlier stage.

A controlled laboratory study was conducted at the USDA/ARS/National Animal Disease Center, Bacterial Diseases of Livestock Research Unit, Ames, IA, USA. The study was carried out in 20 head of Hereford steers sensitized with killed *M. bovis* and compared BOVIGAM™ responses to USA sourced PPD and Pfizer, Australia PPD. Essentially the study showed that positive diagnosis of sensitized cattle occurred in all cattle stimulated with either USA's PPD or Pfizer, Australia's PPD. In addition, studies in New Zealand indicate that the specificity of the assay was not affected by skin testing and it is more sensitive than the Comparative Cervical Skin Test (CCT) when used between 3 and 30 days after the Caudal Fold Skin Test (CFT).

Kit components

Store kit at 35° to 46°F (5±3°C). Bring all reagents except Conjugate Concentrate to room temperature (22±3°C) before use. Return to 35° to 46°F (5±3°C) immediately after use.

Component	Ten (10) Microplate Test Kit (63320) (150 Maximum test samples)	Thirty (30) Microplate Test Kit (63326) (450 Maximum test samples)	Description
1: Microplates coated with antibody to IFN-γ	10 × 96 well plates with lids	30 × 96 well plates with lids	Ready for use.
2: Positive Bovine IFN-γ Control	2 × 1 mL	3 × 2 mL	Contains 0.01% w/v thimerosal. Freeze dried. Reconstitute with deionized or distilled water.
3: Negative Bovine IFN-γ Control	2 × 1 mL	3 × 2 mL	Contains 0.01% w/v thimerosal. Freeze dried. Reconstitute with deionized or distilled water.
4: Green Diluent (Plasma diluent buffer)	1 × 60 mL	1 × 175 mL	Contains 0.01% w/v thimerosal. Ready for use.
5: Wash Buffer – 20x Concentrate	3 × 125 mL	2 × 500 mL	Contains 0.01% w/v thimerosal. Dilute with deionized or distilled water.
6: Conjugate – 100x Concentrate. (Horseradish peroxidase-labelled anti-bovine IFN-γ)	1 × 1.5 mL	2 × 2 mL	Contains 0.01% w/v thimerosal. Freeze dried. Reconstitute with deionized or distilled water.
7A: Blue Diluent (Conjugate diluent buffer – 5x Concentrate)	1 × 25 mL	-	Contains 0.05% w/v thimerosal. Dilute with deionized or distilled water.
7B: Blue Diluent (Conjugate diluent buffer)	-	2 × 175 mL	Contains 0.01% w/v thimerosal. Ready for use.
8: Enzyme Substrate Buffer	1 × 125 mL	2 × 175 mL	Contains H_2O_2. Ready for use.
9: Chromogen Solution – 100x Concentrate	1 × 1.5 mL	2 × 2 mL	Contains TMB in DMSO. Dilute in Enzyme Substrate Buffer.
10: Enzyme Stopping Solution (0.5M H_2SO_4)	1 × 75 mL	1 × 175 mL	Ready for use.

For Veterinary Use Only. For In Vitro Use Only.

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PPD (Protein Purified Derivated)

1998

Tuberculin PPD (Bovine)

Stimulation Antigen for BOVIGAM®

For the laboratory diagnosis of tuberculosis in animals by the Bovine Gamma Interferon Test

Product No.: 63313 **20 ml** © Prionics AG

For *in vitro* veterinary diagnostic laboratory use only.
NOT FOR SKIN TESTING.

Tuberculin PPD (Bovine) is a sterile, preservative free, liquid containing purified protein derivatives prepared from the filtrate of a heat-killed *Mycobacterium bovis* (Strain AN5) grown on synthetic medium. It has been standardised by comparison with the International Standard Preparation of Bovine Tuberculin PPD (Batch 281) and is issued at a strength of 0.3 mg/mL.[1]

GENERAL INSTRUCTIONS

It is essential that all blood samples collected for BOVIGAM® (Bovine Gamma Interferon Test) testing are taken from a cleaned site into a tube containing heparin as anticoagulant. Gently invert tubes several times to ensure complete mixing of blood and heparin. Blood samples should be transported to the testing laboratory at ambient temperature (22°C ± 5°C, avoid extremes) and used in the BOVIGAM® assay within 30 hours of collection.[1]

Blood must not be stored in a refrigerator.

THE BOVIGAM® (BOVINE GAMMA INTERFERON) TEST

BOVIGAM® is a rapid, blood-based assay of cell mediated immunity (CMI) for the diagnosis of bovine tuberculosis in cattle and other ruminants.[1] Tuberculin PPD is mixed with lymphocytes in whole blood and incubated overnight. Blood plasma is then collected from above the cultures and assayed for interferon-γ (IFN-γ) production using the BOVIGAM® enzyme immunoassay (EIA) kit.[2]

USAGE

Mix blood samples evenly immediately before use. For comparative testing, dispense THREE 1.5 mL aliquots of blood into culture vessels. To one aliquot, add 0.1 mL of Tuberculin PPD (Bovine) and mix thoroughly. To the other aliquots add 0.1 mL of sterile PBS to one and 0.1 mL of Tuberculin PPD (Avian) (Prod. No. 63304) to the other. Aseptic procedures should be followed using a sterile pipetting apparatus. Tuberculin PPD (Bovine) may be used directly from a refrigerator. Mix before use.

INTERPRETATION

A positive reaction is indicated by both (1) an increase in the mean OD_{450nm} of the bovine PPD stimulated plasma sample at least 0.05 units greater than that of the BOVIGAM® Negative Control, and (2) when this is also greater than the mean OD_{450nm} for plasmas from Avian PPD- and nil control antigen-stimulated blood. This interpretation algorithm will give maximum test sensitivity.[1]

For New Zealand, a positive reaction is indicated by an increase in mean OD_{450nm} of bovine PPD plasma at least 0.1 units greater than that from both the Avian PPD- and nil control antigen stimulated blood.

RETESTING

Retesting may be performed at any time after last test.

STORAGE

Store, protected from light, at 2° to 8°C. Do not freeze.

MODE OF ISSUE

Tuberculin PPD (Bovine) is available in 20 mL containers

REFERENCES

1. Rothel JS, et al. Aust. Vet. J. 69: 1-4 (1991)
2. Rothel JS, et al. Aust. Vet. J. 67: 134-137 (1990)
3. Jones SL, et al. J. Immunol. Methods 150: 233-240 (1992)
4. Wood PR, et al. Aust. Vet. J. 68: 286-290 (1991)

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Victoria 3043 Australia

Tuberculin PPD is manufactured for:

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Wagistrasse 27a
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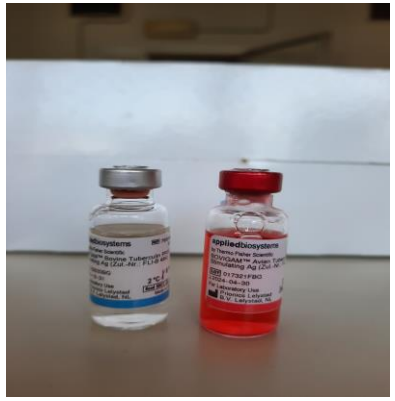
Switzerland

63313RP/C71

2022



appliedbiosystems — INSTRUCTIONS FOR USE

BOVIGAM™ Bovine Tuberculin PPD Stimulating Ag

For the laboratory diagnosis of tuberculosis in animals by the bovine gamma interferon test

Catalog Number 7600060 Pub. No. MAN0017035 Rev. A.0

NOT FOR SKIN TESTING

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at **thermofisher.com**. Wear appropriate protective eyewear, clothing, and gloves.

Introduction

Applied Biosystems™ BOVIGAM™ Bovine Tuberculin PPD Stimulating Ag is a sterile, preservative free, liquid containing purified protein derivatives prepared from the filtrate of a heat-killed *Mycobacterium bovis* (Strain AN5) grown on synthetic medium. It has been standardized by Guinea pig potency testing as described in European Pharmacopoeia and is issued at strength of 30,000 IU/mL.

General Instructions

It is essential that all blood samples collected for BOVIGAM™ TB Kit and BOVIGAM™ 2G TB Kit (bovine gamma interferon tests) testing are taken from a cleaned site into a tube containing heparin as anticoagulant. Gently invert tubes several times to ensure complete mixing of blood and heparin. Blood samples should be transported to the testing laboratory at ambient temperature (22±5°C, avoid extremes) and used in the BOVIGAM™ TB Kit and BOVIGAM™ 2G TB Kit assays within 30 hours of collection.

IMPORTANT! Blood must not be frozen or stored in a refrigerator.

BOVIGAM™ (bovine gamma interferon) tests

BOVIGAM™ TB Kit and BOVIGAM™ 2G TB Kit are rapid, blood-based assays of cell mediated immunity (CMI) for the diagnosis of bovine tuberculosis in cattle and other ruminants. Tuberculin PPD is mixed with lymphocytes in whole blood and incubated overnight at 37°C. Blood plasma is then collected from above the cultures and assayed for Interferon-γ (IFN-γ) production using the BOVIGAM™ TB Kit or BOVIGAM™ 2G TB Kit enzyme immunoassay (EIA).

General precautions

Laboratory safety

National Safety Regulations must be strictly followed.

Usage

For stimulation in 96-well cell culture plates:

Dilute 110 µL of BOVIGAM™ Bovine Tuberculin PPD Stimulating Ag in 890 µL of RPMI medium for final assay concentration of 300 IU/mL. Mix blood samples evenly immediately before use. For comparative testing, dispense **three** 250 µL aliquots of blood into culture vessels. To one aliquot, add 25 µL of diluted stock and mix thoroughly. To the other aliquots add 25 µL of sterile PBS to one and 25 µL of BOVIGAM™ Avian Tuberculin PPD Stimulating Ag (Cat. No. 7600065) to the other. Aseptic procedures should be followed using a sterile pipetting apparatus.

For stimulation in 24-well cell culture plates:

Dilute 160 µL of BOVIGAM™ Bovine Tuberculin PPD Stimulating Ag in 840 µL of RPMI medium for final assay concentration of 300 IU/mL. Mix blood samples evenly immediately before use. For comparative testing, dispense **three** 1.5 mL aliquots of blood into culture vessels. To one aliquot, add 100 µL of diluted stock and mix thoroughly. To the other aliquots add 100 µL of nil control (sterile PBS) to one and 100 µL of BOVIGAM™ Avian Tuberculin PPD Stimulating Ag (Cat. No. 7600065) to the other. Aseptic procedures should be followed using a sterile pipetting apparatus.

Interpretation

(See also the respective Package Inserts of BOVIGAM™ TB Kit and BOVIGAM™ 2G TB Kit for further details.)

Compare the absorbance values of the nil antigen, avian and bovine PPD samples for each animal.

Positive = OD Bovine PPD – Nil Antigen ≥ 0.1; and OD Bovine PPD – Avian PPD ≥ 0.1

Negative = OD Bovine PPD – Nil Antigen < 0.1; or OD Bovine PPD – Avian PPD < 0.1

Retesting

Retesting may be performed at any time after the last test.

Storage

Store, protected from light, at 2–8°C. Do not freeze.

Presentation

BOVIGAM™ Bovine Tuberculin PPD Stimulating Ag is available in 5 mL vials.

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs) also known as MSDSs)

NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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Revision history of Pub. No. MAN0017035 (English)

Rev.	Date	Description
A.0	7 June 2017	New document. Converted the legacy document (Bovigam_Lelystad_Tuberculin_PPD_Bovine_v1.4_a.pdf) to the current document template, with associated updates to the publication number, limited license information, warranty, trademarks, and logos.

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7 June 2017

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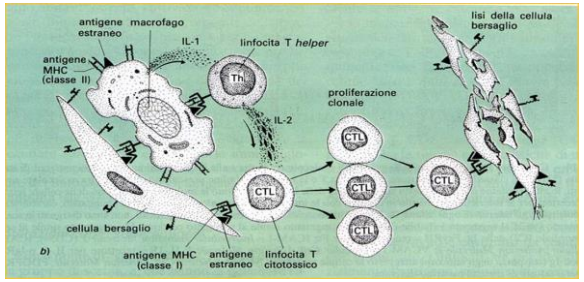
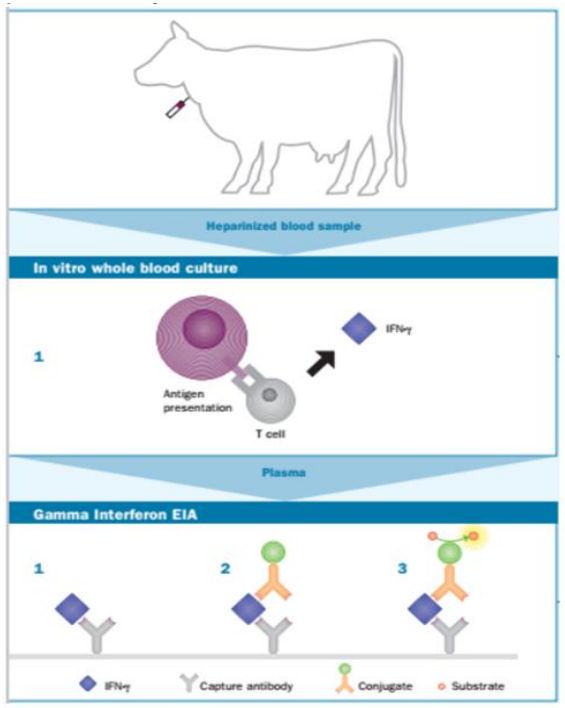


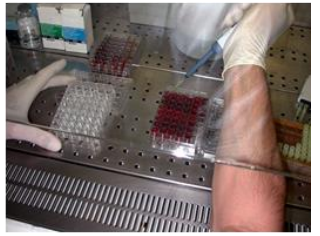


Principio metodica IGRA (Interferon Gamma Release Assay)

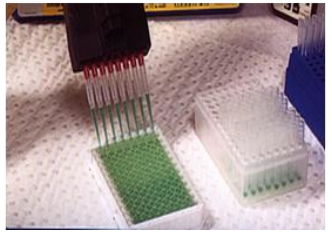
Test diagnostico basato sulla risposta specifica cellulo-mediata cooperazione fra cellula infetta o estranea e linfociti T helper e citotossici.

Il test valuta la capacità dei Linfociti T di sangue periferico di bovino infetto da *Mycobacterium tuberculosis* complex di secernere in vitro linfochine di attivazione (tra cui il gamma-interferon) dopo essere venuti a contatto con gli antigeni .

Sensibilizzazione



ELISA



INDEX

2. MATERIAL

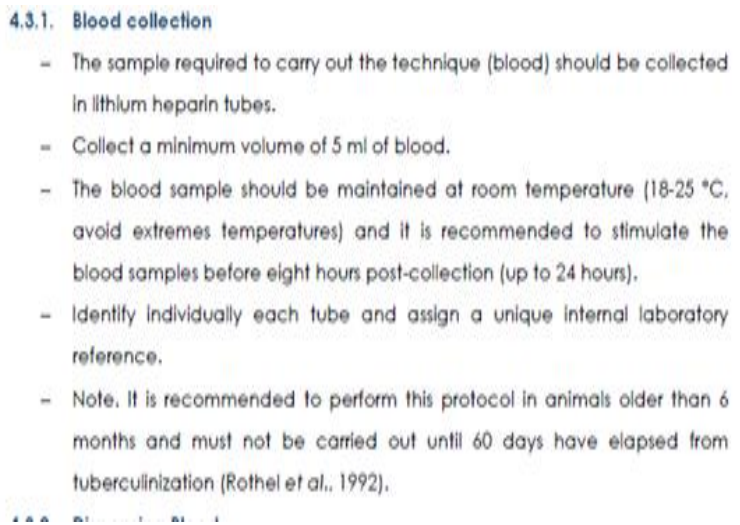
Clinical samples (blood) from the bovine [genera Bos (including the subgenera Bos, Bibos, Novibos, Poephagus) and Bubalus (including the subgenus Anoa) and the offspring of crossings of those species] and caprine animals (species of ungulates belonging to the genus Capra and the offspring of crossings of those species).

4.3.1 BLOOD COLLECTION

Trasporto a temperatura ambiente: (should) 18-25 °C evitando le temperature estreme (kit 19-25 °C con raccomandazione di non portarli sopra i 37°C)

Tempo di stimolazione: raccomandazione prima delle 8 ore dal prelievo (entro le 24 ore).

Nota: Raccomandazione per la categoria di animali da prelevare : animali di età superiore ai 6 mesi e a distanza di almeno 60 giorni dalla prova intradermica (bibliografia1992).



4.3.1. **Blood collection**

- The sample required to carry out the technique (blood) should be collected in lithium heparin tubes.
- Collect a minimum volume of 5 ml of blood.
- The blood sample should be maintained at room temperature (18-25 °C, avoid extremes temperatures) and it is recommended to stimulate the blood samples before eight hours post-collection (up to 24 hours).
- Identify individually each tube and assign a unique internal laboratory reference.
- Note. It is recommended to perform this protocol in animals older than 6 months and must not be carried out until 60 days have elapsed from tuberculinization (Rothel *et al.*, 1992).

Modalità di prelievo e consegna dei campioni di sangue per il test del gamma-interferon per la diagnosi di Tubercolosi Bovina

1. I prelievi possono essere effettuati su tutti i capi dell'azienda con età superiore ai tre mesi.

2. Prelevare da ogni animale il campione di sangue dalla vena giugulare in provetta tipo "**vacutainer" contenente Litio o Sodio Eparina**. Il volume deve essere di almeno 5 ml per provetta. Prima di riporre la provetta, invertirla delicatamente più volte in modo che l'anticoagulante si distribuisca in tutto il campione di sangue.

3. I campione deve essere consegnato, dopo prenotazione telefonica, direttamente al Laboratorio di Biochimica Clinica dell'Istituto Zooprofilattico Sperimentale di Brescia entro 8 ore dal prelievo e non più tardi delle ore 14.00, da lunedì al giovedì.

4. Durante il trasporto I campioni devono essere tenuti a temperatura ambiente.

4.3.3. Addition of antigens

– The optimum concentration for blood stimulation is between 10 and 40 µg PPD/ml of the sample (recommended 20 µg PPD/ml). For example, for a 20 µg PPD/ml and for aliquots of 1.5 ml of blood (24-well plate), prepare a working concentration of avian and bovine PPDs of 0.3 mg/ml (300 µg/ml) (table 3). Aseptically, add 100 µL of either PBS (nil antigen control), avian PPD or bovine PPD to the appropriate 3 wells containing the blood previously dispensed. If necessary, adjust the concentration depending on the available blood volume.

Table 3. Example of preparation of working PPDs concentrations.

	10 blood samples	50 blood samples
Volume required	1 ml	5 ml
Avian PPD* (0.3 mg/ml)	0,6 ml av PPD + 0,4 ml PBS	3 ml av PPD + 2 ml PBS
Bovine PPD* (0.3 mg/ml)	0,3 ml PPD bovina + 0,7 ml PBS	1,5 ml bov PPD + 3,5 ml PBS

* The initial avian and bovine PPD concentration in this example is 0.5 and 1 mg/ml, respectively. These antigens should be prepared to the working PPD concentrations.

Concentrazione ottimale delle Bovine PPD Tuberculin e Avian PPD tuberculin tra 10 e 40 microgrammi/ml di sangue diluite in PBS.

Mitogeno

Note: for checking the viability of the lymphocytes, the blood samples can be stimulated with a mitogen in order to verify the immune state of a group of animals, since they should react by producing gamma-interferon which can be detected by ELISA. It is possible to use pokeweed mitogen at a concentration of 2 µg/ml for sample.

1998

Biologicals (1998) **26**, 225–235
Article No. bg980147

Quality Controls and *in vitro* Diagnostic Efficiency of Bovine PPD Tuberculins

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Abstract. Wide heterogeneity was shown among different batches of bovine protein purified derivative (PPD) tuberculin, as regards protein content and antigenic profile. These features were also investigated in pilot preparations of *Mycobacterium bovis* secreted antigens and PPD tuberculins. Under controlled conditions, widely different compositions were revealed as a function of the *M. bovis* strain and also of the time in culture, due to the transient expression of seemingly important clusters of antigens. Furthermore, these parameters could dramatically affect the efficacy of the above preparations in *in vitro* assays of cell-mediated immunity on *M. bovis*-infected cattle. The field exposure to mycobacteria of the avium/intracellular group could also influence the readout of such assays, results being in agreement with a bystander suppression model of the response to *M. bovis* antigens. Due to the above, practical suggestions are put forward to improve the composition of bovine PPD tuberculins and the relevant control procedures.

Le tubercoline PPD possono essere paragonate solo se:
- Sono allestite con lo stesso ceppo di M. bovis
- Il terreno colturale è identico
- Il tempo di coltura è lo stesso
- La processazione dell'Ag è uguale.

	BOVINE PPD	AVIAN PPD
SOP/004/EIURL	nd	ceppo D4ER
	25000 IU/mL	25000 IU/mL
	1 mg/mL	0,5 mg/mL
	500 IU/mL di sangue (250-1000)	1000 IU/mL di sangue (500-2000)
	20 µg/mL di sangue (10-40)	20 µg/mL di sangue (10-40)
PPD Thermofisher (Spain)	ceppo AN5	ceppo D4ER
	30000 IU/mL	25000 IU/mL
	300 IU/mL di sangue	250 IU/mL di sangIUe
	11 µg/mL sangue	6 µg/mL sangue
	11 µg/mL sangue	7 µg/mL sangue
Officina FarmaceIUtica IZSIUM (Italia)	ceppo AN5	ceppo D4ER
	1 mg/mL	0,5 mg/mL
	50000 IU/mL	25000 IU/mL
	500 IU/mL di sangue	250 IU/mL di sangue
	10 µg/mL sangue	5 µg/mL sangue
AgryqIUality ltd non più commercializzate (Australia)	ceppo AN5	nd
	nd	7500 IU/mL
	300 µg/mL	300 µg/mL
	nd	500 IU/mL di sangue
	20 µg /mL di sangue	20 µg /mL di sangue

	Concentrazione delle PPD bovina e PPD aviare in microgrammi/mL di sangue stimolato									
	20 - 20			10 - 10			5 - 5			11 - 6	
animale	soglia 0,050	soglia 0,100		soglia 0,050	soglia 0,100		soglia 0,050	soglia 0,100		soglia 0,050	soglia 0,100
1	NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO
2	NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO
3	NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO
4	NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO
5	NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO
6	NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO
7	NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO
8	NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO
9	NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO		NEGATIVO	NEGATIVO

4.5.2. Interpretation of results

The interpretation of the results is as follows:

A. **IFN positive:**

OD bov PPD – nil antigen (PBS) ≥ 0.1 and

OD bov PPD– av PPD ≥ 0.1.

B. **IFN negative**: not infected with *M. bovis*/*M. caprae*.

Any of the options not categorized in the previous section.

Note: if the OD av PPD – PBS ≥ 0.1 **and** av PPD OD > bov PPD, an infection by *M. avium*, by other mycobacteria or a recent infection by *M. avium* subsp. *paratuberculosis* could be present.

B. **Inconclusive (when mitogen is used):**

Samples with **an IFN** negative **but** a negative result with the mitogen.

The final report of the results will contain a column showing the following results:

- 'POSITIVE'.
- 'NEGATIVE'.
- 'INCONCLUSIVE'.

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Evaluation of the Specificity of the γ-Interferon Test in Italian Bovine Tuberculosis-free Herds

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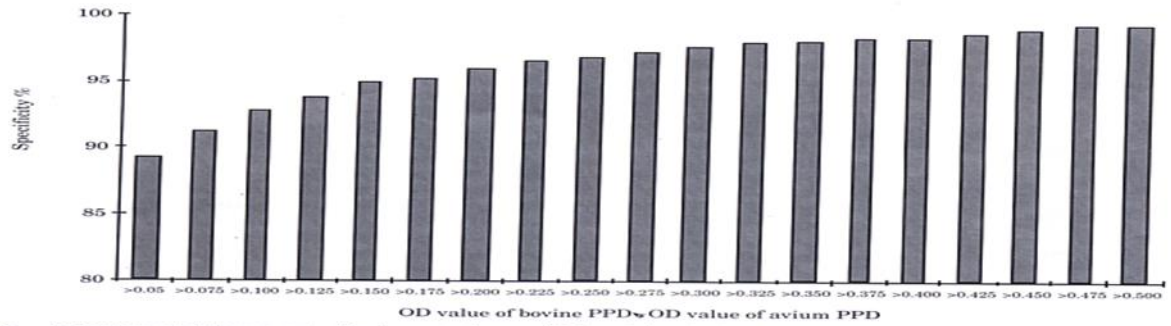

Fig. 5. Influence of different cut-off values on the specificity of the single γ-IFN test.

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VETQAS Proficiency testing for veterinary laboratories

APHA SCIENTIFIC

Results tabulation for 14969/SE PT0065: Gamma interferon (Bovine TB) serum **Distribution Date: 19/04/2022**

ELISA				22/7411		22/7412	
	Kit Manufacturer	Kit Batch Number	Basis for interpretation of final result	Overall OD	Interpretation (+ve/-ve/IC)	Overall OD	Interpretation (+ve/-ve/IC)
Intended				9512		1395	
					+ve		-ve
1119	Bovigam 2G Prionics | ID Screen Rum IFN-g IDVet	10120Z | I85	As by the manufacturers: PPDB-PPDA AND PPDB-NIL > 0.1 = POS | (PPDB-A)/(PC-NC) > 0.35 = POS	2.24 | 220%	+ve	-0.03 | -6%	-ve
1141	Unable to Test						
1602	Prionics Bovigam M. bovis IFN-g Kit	6332615801	Bov-Nil greater than or equal to 0.1 & Bov-Av greater than or equal to 0.1	B-A = 3.239	+ve	B-A = -0.078	-ve
1985	Nil Return						
1998	THERMOFISHER SCIENTIFIC (BOVIGAM)	6332615901	PPDB-PPDA>0.1, PWM =0.45, NIL =0.3	2.874	+ve	-0.026	-ve
2112	Thermofisher Scientific (Bovigam)	6332615401	PPDB-PPDA>0.1, PWM =0.45, NIL =0.3	3.464	+ve	-0.041	-ve
2128	Applied Biosystems	6332616101	B>0.100,B-N>0.05,B-A>0.080	3.369, 2.745	+ve	0.011,-0.036	-ve
2523	LIFE TECHNOLOGIES	6332001601	+ve: BOV OD-NIL OD >= 0.100 and BOV OD-AV OD >=0.100; -ve : any of the options not categorized in the previous section.	3.351	+ve	-0.035	-ve
2535	ThermoFisher	6332616201	OD Bovine PPD -NIL >/= 0.1; OD Bovine PPD -Avian PPD >/= 0.1	2.551	+ve	-0.028	-ve
2824	Thermo Fisher Bovigam	6332001601	POS: (BOV OD / NIL OD) > 2 AND (BOV OD - AV OD) > 0.05	2.491	+ve	-0.019	-ve
2879	Prionics Bovigam	6332615701	POS= B>0.1, B-N>=0.050, B-A>=0.080	3.431, 2.734	+ve	0.005, 0.109	-ve
2895	IDvet, ID Screen Ruminant IFN-γ	I85	EU-RL SOP 006	2.11	+ve	-0.11	-ve

TABLE 1 | IFN-γ test interpretative criteria adopted in the study.

Criteria	INTERPRETATIVE CRITERIA	
Criterion 1	PPDB-PBS ≥ 0.1 and PPDB-PPDA ≥ 0.1 = **POSITIVE**	
	PPDB-PBS < 0.1 = **NEGATIVE**	
	PPDB-PPDA < 0.1 = **NEGATIVE**	
Criterion 2	PPDB ≥ 2*PBS and (PPDB-PPDA) ≥ 0.050 = **POSITIVE**	
	PPDB ≤ 2*PBS = **NEGATIVE**	
	PPDB ≤ PPDA = **NEGATIVE**	
	PPDB ≥ 2*PBS and 0.001 ≤ (PPDB-PPDA) ≤ 0.049 = **INCONCLUSIVE**	
Criterion 3	If the basal value exceeds 0.150 OD before stimulation, the sample is considered UNSUITABLE	
	First level	PPDB and PPDA < 2*PBS = **NEGATIVE**
		PPDB ≥ 2*PBS = **BOVIS**
		PPDA ≥ 2*PBS = **AVIUM**
	If PPDB and PPDA > 2*PBS then do PPDB/PPDA	If PPDB/PPDA ≤ 0.9 = **AVIUM**
		PPDB/PPDA ≥ 1.1 = **BOVIS**
		0.9 < PPDB/PPDA <1.1 = **INCONCLUSIVE (IN)**
	Second level	If Lely PPDs = Bovis and It PPDs = Bovis then **POSITIVE**
		If Lely PPDs = Negative and It PPDs = Negative then **NEGATIVE**
		If Lely PPDs = Avium/Neg and It PPDs = Avium/Neg then **NEGATIVE**
		If Lely PPDs = IN/A/Neg and It PPDs = Bovis then **Not Discriminant (ND)**
		If Lely PPDs = Bovis and It PPDs = IN/A/Neg then **Not Discriminant (ND)**
Criterion 4	ESAT6/CFP10-PBS ≥ 0.1 = **POSITIVE**	
	ESAT6/CFP10-PBS < 0.1 = **NEGATIVE**	

The bold values are the results of IFN-γ assays according to 4 criteria
PPD, Purified Protein Derivative; PPDB, Bovine PPD; PPDA, Avian PPD; Lely, Lelystad PPDs; and It, Italian PPDs.

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OIE-register-bovigam-abstract-v1-05-2015

OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

Name of the diagnostic kit: BOVIGAM®, Mycobacterium bovis Gamma interferon test kit for cattle
Manufacturer: Prionics AG
OIE Approval number: 20150110
Date of Registration: May 2015

Disease: Bovine Tuberculosis

Pathogen Agent: Mycobacterium bovis and other mycobacteria belonging to the tuberculosis complex (e.g. M. caprae)

Type of Assay: Sandwich ELISA

Purpose of Assay: Certified by the OIE fit for the detection of cell mediated immune response to infection with Mycobacterium bovis and other mycobacteria belonging to the tuberculosis complex on analysis of whole blood specimens in cattle, buffalo (Syncerus caffer), goat and sheep (provisionally) for the following purposes:

1. Historical freedom
2. Re-establishment of freedom after outbreaks
3. Certify freedom from infection or agent in individual animals or products for trade/movement purposes
4. Eradication of infection from defined populations
5. Confirmatory diagnosis of suspect or clinical cases (includes confirmation of positive screening test)
6. Estimate prevalence of infection to facilitate risk analysis (surveys/herd health schemes/disease control)
7. Ancillary test for eradication of Tuberculosis

Species and Specimen: Cattle, Buffalo (Syncerus caffer), Goats and Sheep (provisionally) - blood-based in vitro laboratory test

1. Information on the kit

Please refer to the kit insert available on the OIE Registry web page or contact manufacturer at:

Website link: http://www.prionics.com/tuberculosis/

Email address: info@prionics.com

Abstract Sheet – BOVIGAM®, Mycobacterium bovis Gamma interferon test kit for cattle
Version 1 – June 2015
Page 1 of 3

Criterion 1: BOD_COD > 0 and BOD_AOD > 0;
Criterion 2: BOD/COD > 1.25 and BOD_AOD > 0;
Criterion 3: BOD/COD > 1.5 and BOD_AOD > 0;
Criterion 4: BOD_COD P0.05 and BOD_AOD > 0;
Criterion 5: If BOD = 0.1, then BOD/COD > 1.5 and BOD_AOD > 0. If BOD > 0.1, then BOD_COD > 0.05 and BOD_AOD > 0;
Criterion 6: BOD_AODP0.1;
Criterion 7: BOD_COD P0.1 and BOD/AODP 1.8;
Criterion 8: BOD_COD P0.1 and BOD/AODP 1.25;
Criterion 9: BOD/AODP1.8;
Criterion 10: BOD_COD P0.05 and BOD/AODP 1.8 ("criterion 4 if BOD/AODP1.0);
Criterion 11: BOVIGAM®: BOD_COD P0.1 and BOD_AOD > 0;
Criterion 12: BOD_COD P2(COD) and BOD_AODP0.05;
Criterion 13: BOD_COD P0.1 and BOD_AODP 0.1;
Criterion 14: BOD_AODP0.04.
BOD: Mean optical density value of the plasma from the bovine PPD-stimulate blood.
AOD: Mean optical density value of the plasma from the avian PPD-stimulated blood.
COD: Mean optical density value of the plasma from blood incubated with phosphate buffered saline (nil antigen control).

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VISAVET HEALTH SURVEILLANCE CENTRE
COMPLUTENSE UNIVERSITY OF MADRID

European Union Reference Laboratory
for Bovine Tuberculosis

Validation report

SOP/004/EURL

DIAGNOSIS OF TUBERCULOSIS INFECTION IN BOVINE AND CAPRINE ANIMALS FOR USING THE IN VITRO GAMMA-INTERFERON DETECTION ASSAY

Applied Biosystems™ BOVIGAM™ TB Kit

Revision 0 (19/04/2021)

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Species and Specimen: Cattle, Buffalo (*Syncerus caffer*), Goats and Sheep (provisionally) - blood-based in vitro laboratory test

1. Information on the kit

Please refer to the kit insert available on the OIE Registry web page or contact manufacturer at:

Website link: http://www.prionics.com/tuberculosis/

Email address: info@prionics.com

REPORT DI VALIDAZIONE

4. Expected specifications

Table 1 defines the expected specifications defined by the manufacturer, according to the abstract sheet of the OIE Procedure for Registration of Diagnostic Kits by the OIE.

Table 1. Expected specifications provided by the manufacturer.

Analytical specificity	No detection of recombinant IFN- α γ β. Positive reactivity to avian and bovine PPD stimulated blood samples from animals infected with members of the *Mycobacterium tuberculosis* complex
Analytical sensitivity	80 pg/ml of recombinant bovine IFN-γ
Repeatability (within plate)	Coefficient of variation <10%
Repeatability (between plates)	Coefficient of variation <10%
Reproducibility	<15%
Diagnostic specificity	Cattle: Classical statistics with PPDs 97.4% (95%CI = 87.5-99.6%)
Diagnostic sensitivity	Cattle: Classical statistics with PPDs 84.6% (95%CI = 73.0-95.5%)

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REPORT DI VALIDAZIONE

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Annex

Table 2. Repeatability within plate using the Bovigam batch number 6332614801.

Day	Plate position	Sample ID	OD PBS	OD aPPD	OD bPPD	IFNbov*
08/03/2021	G7-G8-G9	VV-E-1616	0.0638	0.0725	0.4400	0.3762
08/03/2021	H7-H8-H9	VV-E-1616	0.0589	0.0650	0.4106	0.3517
08/03/2021	A10-A11-A12	VV-E-1616	0.0581	0.0638	0.4012	0.3431
08/03/2021	B10-B11-B12	VV-E-1616	0.0632	0.0675	0.3851	0.3219
08/03/2021	C10-C11-C12	VV-E-1616	0.0603	0.0694	0.4147	0.3544
08/03/2021	D10-D11-D12	VV-E-1616	0.0688	0.0634	0.4041	0.3353
08/03/2021	E10-E11-E12	VV-E-1616	0.0582	0.0578	0.4163	0.3581
08/03/2021	F10-F11-F12	VV-E-1616	0.0609	0.0615	0.4189	0.3580
08/03/2021	G10-G11-G12	VV-E-1616	0.0670	0.0609	0.4208	0.3538
08/03/2021	H10-H11-H12	VV-E-1616	0.0581	0.0544	0.4272	0.3691
Mean			0.0617	0.0636	0.4139	0.3522
Standard Deviation			0.0039	0.0054	0.0150	0.0157
CV (%)			6.2452	8.4496	**3.6329**	**4.4640**

*IFNbov value = OD bPPD – OD PBS

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Table 3. Reproducibility using the Bovigam batch number 6332614801.

Day	Plate position	Sample ID	OD PBS	OD aPPD	OD bPPD	IFNbov*
27/11/2020	F1-F2-F3	VV-E-1616	0.1184	0.0908	0.3567	0.2383
27/11/2020	G1-G2-G3	VV-E-1616	0.1038	0.0807	0.3576	0.2538
27/11/2020	H1-H2-H3	VV-E-1616	0.0780	0.0695	0.3531	0.2751
27/11/2020	A4-A5-A6	VV-E-1616	0.0677	0.0726	0.3679	0.3002
27/11/2020	B4-B5-B6	VV-E-1616	0.0701	0.0710	0.3700	0.2999
27/11/2020	C4-C5-C6	VV-E-1616	0.0688	0.0640	0.3578	0.2890
27/11/2020	D4-D5-D6	VV-E-1616	0.0667	0.0638	0.3501	0.2834
27/11/2020	E4-E5-E6	VV-E-1616	0.0447	0.0482	0.2695	0.2248
27/11/2020	F4-F5-F6	VV-E-1616	0.0703	0.0692	0.3617	0.2914
27/11/2020	G4-G5-G6	VV-E-1616	0.0632	0.0603	0.3510	0.2878
12/02/2021	E1-E2-E3	VV-E-1616	0.0762	0.0734	0.3451	0.2689
12/02/2021	F1-F2-F3	VV-E-1616	0.0862	0.0807	0.3355	0.2493
12/02/2021	G1-G2-G3	VV-E-1616	0.0839	0.0812	0.3356	0.2517
12/02/2021	H1-H2-H3	VV-E-1616	0.0887	0.0751	0.3546	0.2659
12/02/2021	A4-A5-A6	VV-E-1616	0.0965	0.1164	0.3962	0.2997
12/02/2021	B4-B5-B6	VV-E-1616	0.0838	0.0887	0.3465	0.2627
12/02/2021	C4-C5-C6	VV-E-1616	0.0744	0.0819	0.3486	0.2742
12/02/2021	D4-D5-D6	VV-E-1616	0.0719	0.0725	0.3413	0.2694
12/02/2021	E4-E5-E6	VV-E-1616	0.0840	0.0816	0.3579	0.2739
12/02/2021	F4-F5-F6	VV-E-1616	0.0837	0.0835	0.3776	0.2939
08/03/2021	G7-G8-G9	VV-E-1616	0.0638	0.0725	0.4400	0.3762
08/03/2021	H7-H8-H9	VV-E-1616	0.0589	0.0650	0.4106	0.3517
08/03/2021	A10-A11-A12	VV-E-1616	0.0581	0.0638	0.4012	0.3431
08/03/2021	B10-B11-B12	VV-E-1616	0.0632	0.0675	0.3851	0.3219
08/03/2021	C10-C11-C12	VV-E-1616	0.0603	0.0694	0.4147	0.3544
08/03/2021	D10-D11-D12	VV-E-1616	0.0688	0.0634	0.4041	0.3353
08/03/2021	E10-E11-E12	VV-E-1616	0.0582	0.0578	0.4163	0.3581
08/03/2021	F10-F11-F12	VV-E-1616	0.0609	0.0615	0.4189	0.3580
08/03/2021	G10-G11-G12	VV-E-1616	0.0670	0.0609	0.4208	0.3538
08/03/2021	H10-H11-H12	VV-E-1616	0.0581	0.0544	0.4272	0.3691
Mean			0.0733	0.0720	0.3724	0.2992
Standard Deviation			0.0154	0.0131	0.0366	0.0427
CV (%)			21.0378	18.2009	**9.8174**	**14.2887**

*IFNbov value = OD bPPD – OD PBS

REPORT DI VALIDAZIONE

5. Evaluation in the laboratory

These are the parameters evaluated in the laboratory by the EU-RL:

5.1. Repeatability

Repeatability was evaluated with an IFN-γ internal reference material from cattle. It was evaluated in one batch, in the same plate and with 10 replicates of positive reference material. The coefficient of variation at the Optical Density at the bovine well and the bovine IFN value (bovine OD -PBS OD) was 3.63 and 4.46%, respectively (Annex, Table 2). Therefore, it is in accordance with the manufacturer validation.

5.2. Reproducibility

Reproducibility was evaluated with an IFN-γ internal reference material from cattle. It was evaluated in a unique batch in different days and analysts, and with a total of 30 replicates. The coefficient of variation at the Optical Density at the bovine well and the bovine IFN value (bovine OD -PBS OD) was 9.82 and 14.29%, respectively (Annex, Table 3). Therefore, it is in accordance with the manufacturer validation.

5.3. Repeatability of stimulation in 24 or 96-well plates

One hundred and thirty-seven (n=137) blood samples were stimulated in 24 and 96-well plates. When available, 1.5ml of blood was used in the 24-well plates and always 250 µl of blood in the 96 well plates. The final concentration of PPDs was 20µg/ml.

The cut-off used was the one recommended by the manufacturer (OD bovine PPD – PBS≥0.1 and OD bovine – avian PPD≥0.1). The kappa agreement was 0.88 (excellent). The two samples with discrepant results were close to the cut-off (Annex, Table 4).

5.4. Diagnostic sensitivity

Bos taurus

Under evaluation with a high number of samples using the interpretation criterium and the cut-off recommended by the manufacturer (OD bovine PPD – PBS≥0.1 and OD bovine – avian PPD≥0.1) that was approved by all the NRLs for Bovine Tuberculosis in 2015.

***Bubalus* spp.**

Data provided in this document were extracted from the Martucciello *et al.*, 2020 publication.

Sensitivity of the IFN-gamma test was evaluated in a population of 489 buffaloes from 71 herds (range from 1-35 animals) of confirmed bovine TB outbreaks. All the animals were previously positive to the single intradermal tuberculin test and, 42 days after, the SIT the CIT and blood sampling for IFN-gamma test was done. The positive animals to CIT and/or IFN were slaughtered, and organs were examined for TB-compatible lesions and cultured. In case of infected herds, an animal was considered positive if bovine TB lesions were found at the slaughterhouse and/or proved to be positive on the culture test and/or direct PCR on tissue samples.

The blood samples were stimulated within the 8h after collection, using bovine and avian PPD, PBS as a control, and the pokeweed mitogen to assure the viability and response of lymphocytes. Plasma samples were preserved frozen until use.

The cut-off included in this validation report is the one recommended by the manufacturer (OD bovine PPD – PBS≥0.1 and OD bovine – avian PPD≥0.1) that is the same approved by all the NRLs for Bovine Tuberculosis in 2015.

Diagnostic sensitivity according to this criterium was 94.7% (IC 95%, 92.3–96.5). Considering only the animals with conclusive results in all the evaluation criteria included in this Italian study (n=434), the diagnostic sensitivity increased to 97.7 (IC 95%, 95.8–98.9) (Annex, Table 5).

The specificity in 95 animals from the farm with paratuberculosis was 98.9 (IC 95%, 99.9–94.3). Therefore, it is in accordance with the manufacturer validation.

Caprine animals

Data provided in the present document was extracted for the meta-analysis performed by Roy and collaborators (2020). Cut-off recommended in goats is the same previously described for cattle in the present report ((OD bovine PPD – PBS≥0.1 and OD bovine – avian PPD≥0.1). The global pooled sensitivity achieved using this interpretation criterium was 66% (IC 95%, 36-87). A sensitivity of 72% (IC 95%, 21-96.3) was reported in paratuberculosis-free animals and 78% (IC95%, 51-93) in non-paratuberculosis vaccinated animals, decreasing significantly in the presence of paratuberculosis infection or vaccination [58% (IC95%, 49-66) and 26% (IC95%, 19-35).

5.5. Diagnostic specificity

Bos taurus

A total of 1,697 samples from cattle located in 2 Galicia and Castilla y Leon from beef and dairy cattle were included for this analysis. The cut-off used was the one recommended by the manufacturer (OD bovine PPD – PBS≥0.1 and OD bovine – avian PPD≥0.1) that is the one approved by all the NRLs for Bovine Tuberculosis in 2015.

The samples included in this study were stimulated within the 8h after collection, using bovine and avian PPD, PBS as a control, and the pokeweed mitogen to assure the viability and response of lymphocytes. Plasma samples were preserved at - < -15ºC until use.

Seventy animals out of 1,697 showed a positive result and the diagnostic specificity according to this criterium was 95.9 (IC 95%, 94.8–96.7). The values are included in the range defined by the manufacturer but an additional set of samples from other Member States will be evaluated.

Bubalus bubalis

Data provided in this document were extracted from the Martucciello *et al.*, 2020 publication.

Specificity of the IFN-gamma test was evaluated in a population of 458 buffaloes from four officially tuberculosis free (OTF) herds. Additionally, 95 animals from an OTF farm with paratuberculosis was included in this study, and the Single Intradermal Tuberculin Test was also performed at the same time of blood collection.

The blood samples were stimulated within the 8h after collection, using bovine and avian PPD, PBS as a control, and the pokeweed mitogen to assure the viability and response of lymphocytes. Plasma samples were preserved frozen until use.

The cut-off included in this validation report is the one recommended by the manufacturer (OD bovine PPD – PBS≥0.1 and OD bovine – avian PPD≥0.1) that is the same approved by all the NRLs for Bovine Tuberculosis in 2015.

Diagnostic specificity according to this criterium was 98.5 (CI 95%, 98.5–96.9). The specificity in 95 animals from the farm with paratuberculosis was 98.9 (CI 95%, 99.9–94.3) (Annex, Table 6). Therefore, it is in accordance with the manufacturer validation.

Caprine animals

Data provided in the present document was extracted for the meta-analysis performed by Roy and collaborators (2020). Cut-off recommended in goats is the same previously described for cattle in the present report ((OD bovine PPD – PBS≥0.1 and OD bovine – avian PPD≥0.1). The global pooled specificity achieved using this interpretation criterium was 99% (IC 95%, 95-100) decreasing to 93% (IC 95%, 95-100) in paratuberculosis-vaccinated goats.

5.6. Conclusion

This validation report certifies the validation of the Applied Biosystems™ BOVIGAM™ TB Kit (THermofisher Scientific) for the samples of interest and purpose described above.

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Procedura generale per la validazione dei metodi sierologici
Metodi normati

Metodi normati

Accordanza

L'Accordanza è la probabilità di ottenere lo stesso risultato da due identiche prove effettuate sullo stesso campione (entrambe positive o entrambe negative).

Concordanza

La Concordanza è la probabilità di ottenere lo stesso risultato da due identiche prove effettuate sullo stesso campione in condizioni di riproducibilità.

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Titolo del metodo di prova normato

METODO DI PROVA NORMATO PER LA DIAGNOSI DI TUBERCOLOSI NEL SANGUE BOVINO E CAPRINO MEDIANTE RICERCA DI INTERFERON-GAMMA SAGGIO IGRA (INTERFERON GAMMA RELEASE ASSAY)–APPLIED BIOSYSTEMS™ BOVIGAM™ TB KIT (THERMO FISHER SCIENTIFIC)

Norma di riferimento

SOP/004/EURL «**DIAGNOSIS OF TUBERCULOSIS INFECTION IN BOVINE AND CAPRINE ANIMALS FOR USING THE IN VITRO GAMMA-INTERFERON DETECTION ASSAY.APPLIED BIOSYSTEMS™ BOVIGAM™ TB KIT (THERMO FISHER SCIENTIFIC)»**
European Union Reference Laboratory for Bovine Tuberculosis ,VISAVET HEALTH SURVEILLANCE CENTRE, COMPLUTENSE UNIVERSITY OF MADRID

Grazie dell’attenzione