

ICS 11.220  
CCS B 41



**NATIONAL STANDARD OF THE  
PEOPLE'S REPUBLIC OF CHINA**

**GB/T 32945-2016**

---

**Bovine tuberculosis diagnosis -- Assay on IFN- $\gamma$  in vitro**

牛结核病诊断 体外检测 $\gamma$ 干扰素法

**(English Translation)**

**Issued on: August 29, 2016**

**Implemented on: March 1, 2017**

---

**Issued by: General Administration of Quality Supervision, Inspection and  
Quarantine of the People's Republic of China**

## Table of Contents

|                                                                   |
|-------------------------------------------------------------------|
| Preface                                                           |
| Introduction                                                      |
| 1 Scope                                                           |
| 2 Abbreviations                                                   |
| Principle                                                         |
| 4 IFN-gamma in vitro assay                                        |
| 4.1 Instruments and supplies                                      |
| 4.2 Reagent                                                       |
| 4.3 Procedure                                                     |
| Appendix A (normative) Reagent preparation                        |
| Appendix B (informative) Sampling and sample handling precautions |

## Foreword

This standard is drafted in accordance with the rules given in GB/T 1.1-2009.

## Introduction

Bovine tuberculosis is mainly caused by *Mycobacterium bovis* (*Mycobacterium bovis*, M. bovis) caused by a zoonotic chronic infectious diseases, Showing a worldwide distribution. The World Organization for Animal Health (OIE) lists it as a must-report and international trade must be classified as Second-class animal disease, is the "national long-term animal disease prevention and control plan (2012 ~ 2020)" 16 kinds of priority control of the domestic major animal disease one. Clinical tuberculosis is the most common type of tuberculosis, but breast tuberculosis and intestinal tuberculosis can also occur. Healthy cattle can be contaminated by Air, feed and drinking water through the respiratory and gastrointestinal infections; In addition, cattle tuberculosis is one of the important sources of human tuberculosis. As the cattle branch Bacteria infection spectrum widely, persistent infection, cattle slow course of tuberculosis, clinical symptoms such as atypical reasons, cattle tuberculosis purification is relatively difficult. along with in recent years, China's modern dairy industry and the rapid development of beef cattle industry and domestic cattle trade increasingly frequent, China's cattle tuberculosis is on the rise.

Domestic and foreign generic bovine tuberculosis control strategy is "quarantine - culling", therefore, accurate detection is the premise of control and purification of bovine tuberculosis.

The current quarantine method for bovine tuberculin intradermal allergy, the method has the advantage of high sensitivity, the disadvantage is. the operation time-consuming cumbersome, knot Fruit judgment is subjective and can be cross-reactive with environmental mycobacterial infections, immunosuppressive or tuberculosis reactivity to tuberculin reduce. Bovine tuberculosis diagnosis in vitro detection of IFN-gamma method is the use of bovine tuberculin and tuberculin to stimulate peripheral blood lymphocyte release IFN-gamma, a method for determining the infection status of bovine tuberculosis by detecting IFN-gamma concentration is a method with high sensitivity and specificity, and can achieve high-throughput detection. Developed countries have successfully used this method to assist in the diagnosis and monitoring of bovine tuberculosis. Establishment of China 's Bovine Tuberculosis Diagnosis IFN-gamma in vitro test national standard, for cattle tuberculosis early diagnosis, quarantine, monitoring and epidemiological survey to provide technical support, to promote Into the control and purification of bovine tuberculosis in China, and ensure the healthy and sustainable development of cattle industry in China.

In vitro detection of bovine tuberculosis by interferon - gamma method

## 1 Scope

This standard specifies the abbreviations, principles, reagents, sampling and operating procedures for the detection of gamma tuberculosis in vitro by the detection of bovine tuberculosis.

This standard applies to the early diagnosis, quarantine, surveillance and epidemiological investigation of bovine tuberculosis.

## 2 Abbreviations

The following abbreviations apply to this document.

A-PPD. avianpurifiedproteinderivative B-PPD. bovinepurifiedproteinderivative ELISA. enzyminked immunosorbent assay (enzymelinkedimmunosorbentassay) HRP. horseradishperoxidase IFN-gamma. interferonamma IgG. immunoglobulin G PBS. phosphate buffer (phosphatebuffersolution) OD. optical density

## 3 Principle

Mycobacterium tuberculosis positive tuberculosis, its peripheral blood lymphocytes have been Mycobacterium bovis sensitized and produce immune memory. When in vitro in the event of bovine mycobacterial specific antigen (bovine tuberculin) stimulation, lymphocytes will be activated, the occurrence of re-cellular immune response, Put IFN-gamma. Mycobacterium bovis infection can be diagnosed by detecting the level of IFN-gamma by sandwich ELISA. Mycobacterium tuberculosis is non-tuberculosis Mycobacterium tuberculosis, with tuberculin to stimulate lymphocytes as a control, can effectively rule out

the environment caused by mycobacterial infection nonspecific reaction.

#### **4.1 Instruments and supplies**

##### **4.1.1**

Class II biological safety cabinet.

##### **4.1.2**

Carbon dioxide incubator.

##### **4.1.3**

Microplate reader.

##### **4.1.4**

Mixer.

##### **4.1.5**

refrigerator.

##### **4.1.6**

balance.

##### **4.1.7**

high pressure disinfection pot.

##### **4.1.8**

trace adjustable pipette and tip.

##### **4.1.9**

Disposable sterile centrifuge tube (2 mL).

##### **4.1.10**

disposable heparin sodium anticoagulant blood collection tube.

##### **4.1.11**

Disposable syringes (5 mL).