UNOFFICIAL TRANSLATION

THAI AGRICULTURAL STANDARD

TAS 10001-2004

DIAGNOSTIC TEST OF BOVINE TUBERCULOSIS

National Bureau of Agricultural Commodity and Food Standards
Ministry of Agriculture and Cooperatives
50 Phaholyothin Road, Ladyao, Chatuchak, Bangkok 10900
Telephone (662) 561 2277 www.acfs.go.th

Published in the Royal Gazette Vol.121 Section 120D,
dated 22 October B.E. 2547 (2004)
Standard for the diagnosis of bovine tuberculosis is provided for the precision and accuracy of animal diagnostic laboratory.

This standard was originally drafted by the Department of Livestock Development and it was revised by the Committee of Animal Sanitary Standards and finally approved by the National Committee on Agricultural Commodity and Food Standards.

The establishment of this standard is based on the information of the following documents:


**Remark:**

The standard title has been revised from “Thai Agricultural Commodity and Food Standard (TACFS)” to “Thai Agricultural Standard (TAS)” in accordance with the enforcement of The Agricultural Standards Act B.E. 2551 (2008)
The resolution of the 1/2547 session of the National Committee on Agricultural Commodity and Food Standards dated 15 March B.E.2547 (2004) endorsed the Thai Agricultural Commodity and Food Standard entitled Diagnostic Test for Bovine Tuberculosis. This standard would be of benefits for quality improvement, facilitating trade and protecting consumers.

By virtue of the Cabinet Resolution on Appointment and Authorization of the National committee on Agricultural Commodity and Food Standards dated 19 November B.E.2545 (2002), the Notification on Thai Agricultural Commodity and Food Standard entitled Diagnostic Test for Bovine Tuberculosis is hereby issued as voluntary standard, the details of which are attached herewith.

Notified on 3 August B.E.2547 (2004)

Mr. Somsak Thepsuthin

Minister of Agriculture and Cooperatives
Chairperson of the National Committee on Agriculture Commodity and Food Standards
THAI AGRICULTURAL STANDARD
DIAGNOSTIC TEST FOR BOVINE TUBERCULOSIS

1. INTRODUCTION

Bovine tuberculosis is caused by Mycobacterium bovis. The predominant lesion is small nodule so-called tubercle. Bovine tuberculosis is mostly chronic disease, however, the severe acute form can be occasionally found. Lesions can be found in several tissues, even though they are frequently seen in lymph nodes at head and neck, lung, intestines, liver and pleural cavity. Generally, intradermal test is the most common test for the diagnosis in the live animal on the basis of delayed hypersensitivity reaction to bovine tuberculin. Interpretation of intradermal test can be done 72 hours after injection. This method is an international standard to certify bovine tuberculosis for cattle trade. Intradermal test is one of several methods which is based on delayed type hypersensitivity reaction. Delayed type hypersensitivity or type IV reaction (Coombs and Gell, 1975) is usually occurred 24-48 hours after antigen get into the body which is the reaction of specifically sensitized T cell lymphocytes to antigen and resulting in the release of lymphokines. There is inflammation and accumulation of mononuclear cells at the injection site.

The mechanism of type IV reaction at the antigen injection site (purified protein derivative tuberculin, PPD) is the rapid distribution of antigen from the injection site and there is only 10-20% of the antigen left after 5 hour post-injection (h.p.i.) due to phagocytosis by macrophages. At this stage, there is only little inflammation and there is no different between normal and hypersensitized animals. Later on, the injection site becomes induration and erythema which is maximized at 24-48 h.p.i. and most of the infiltrated cells are lymphocytes and macrophages infiltrating at this site. At the early stage of inflammation, the sensitized T cells exposed with the antigen will release the lymphokines. These lymphokines i.e. macrophage migratory inhibition factor: MIF inhibit migration of macrophages, but MIF has no effect on migration of polymorphonuclear cells. Moreover, the release of skin reactive factor (SRF) stimulates local inflammation and increase permeability of the blood vessels.

2. DIAGNOSIS

Intradermal test is the standard method for the diagnosis of tuberculosis in live bovine. In the past, heat-concentrated synthetic medium (HCSM) tuberculin was preferred to use as a diagnostic reagent; however, nowadays it has been replaced by PPD tuberculin because it is more specific and widely used as well as commercially available. For the diagnosis of bovine tuberculosis, single intradermal test using bovine tuberculin or intradermal comparative test using avian tuberculin combined with bovine tuberculin can be used for the test. The preferred position for intradermal test is mid-neck as it is more sensitive than the caudal fold of tail. However, the caudal fold of tail can be applied by increasing the amount of the reagent.
2.1 MATERIALS AND REAGENTS

1) PPD tuberculin (bovine): It is the sterilized extract from culture of Mycobacterium bovis (strain AN5) which are grown on artificial medium and autoclaved. The concentration is in mg/ml (Weybridge reference standard).

2) Tuberculin syringe: Tuberculin syringe shall have enough strength and can be sterilized by boiling. The test volume is at least 0.1 ml.

3) Needle: The gauge of the needle is crucial. The needle shall not be too wide and too long because it affects the volume of the reagent. In general, the 26 gauge (0.45 mm in diameter) that have 3-4 mm in length is preferred.

4) Callipers which are device for measurement of skin thickness.

5) All of the instruments using for the diagnosis shall be sterilized before used and shall be free of chemical or antiseptic.

6) Reagents shall be kept at 2-8 °C and shall be protected from light. Do not freeze.

2.2 METHOD

Intradermal test is preformed as follows:

2.2.1 Single intradermal test

Reagent of 0.1 ml (2,000 IU) is injected into the caudal fold of the tail. The volume of the reagent shall not exceed 0.2 ml as it is convenience for operation and international accepted. The injection sites shall be clipped and cleaned, but chemicals or antiseptics shall not be used. After injection, the right position is confirmed by observing or palpating a small pea-like swelling at each site of injection. In certain case, it may be necessary to measure the thickness of the skin by callipers before injection.

2.2.2 Intradermal comparative test

The amount of bovine tuberculin and avian tuberculin shall not be less than 2000 IU. The position for injection of both reagents shall be 12-15 cm apart on the same site of the mid-neck. Before test, the injection site shall be marked and measured the thickness with callipers. In young animal in which there is no room to separate the sites sufficiently on one side of the neck, injection shall be made on each side of the neck at identical position in the centre of the middle third of the neck.

3. RESULTS

3.1 The result shall be read at 72 hours post injection. The result is positive if there is a swelling at the injection site. The swelling appearance may be different case by case such as hard, soft, round shape, high rim, diffuse or extensive edema, exudation, necrosis and inflammation etc. Criteria for measuring the swelling are as follows:
Increasing skin thickness  
<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 2 mm</td>
</tr>
<tr>
<td>Between 2-5 mm</td>
</tr>
<tr>
<td>More than 5 mm</td>
</tr>
</tbody>
</table>

3.2 Changes or inflammation on the skin shall be closely observed for further conclusion. For the endemic herd, if there is swelling of the skin by observation or palpation, interpreter shall consider positive.

3.3 Animals that are inconclusive shall be subjected to another test at least 60 days after the previous test

4. GUIDELINES FOR THE DIAGNOSIS OF BOVINE TUBERCULOSIS

4.1 Before testing, the study on the protocol shall be done thoroughly.

4.2 Operating with honesty and responsibility.

4.3 Operator shall read the result by oneself.

4.4 Any chemical or disinfectants for the test not allowed.

4.5 If blood sample needs to be collected, it shall be done before testing.

4.6 If blood sample needs to be collected from the caudal vein, the veterinarian shall stop bleeding completely to prevent swelling which may affect the interpretation of the test.

4.7 If another test needs to be performed, it shall be done at least 60 days after the previous test.

4.8 The records on farm and animal history shall be kept completely and continuously.

4.9 If there are more than 1 injection during the test, the total amount of the reagent shall not more than 0.2 ml.

4.10 If the veterinarian accidentally punctures himself/herself, he/she shall bleed himself/herself as much as possible and clean the puncture wound to prevent infection and tissue necrosis.