

Procedure of tuberculin skin testing (TST)

Commercial purified protein derivative (PPD) produced from *Mycobacterium bovis* (PPD-B) and *Mycobacterium avian* (PPD-A) are used in this test. Before test, PPD-B and PPD-A are diluted in sterilized PBS at 25,000 IU in 1mL.

1. Single cervical test (SCT)

- Firstly, cattle is fixed well, and at the injection site of neck, the feather is shaved off with hair clipper and disinfected with 75% alcohol and iodine tincture .



- Skin thickness is measured with calliper.



- Using a 1ml syringe to aspirate the diluted PPD-B solution, then injecting intradermally 0.1 mL PPD-B (at least 2,000 IU) to cervical skin.



- After 72 hours, observing whether there is an inflammatory reaction at the injection site. The skin thickness at the injection site is measured again. The difference of skin thickness between two measurements is calculated.



- Interpretation of result. The obvious swelling of the skin at the injection site can be regarded as positive for bovine tuberculosis. Additionally, skin thickness difference of less than 2 millimeters is considered negative, skin thickness difference between 2-4 millimeters is considered suspicious, and a difference in skin thickness greater than 4 millimeters is considered positive. Suspicious and negative animals should be measured again after 96 h and 120 h.



ΔB	Interpretation of result
$\leq 2\text{mm}$	Negative
$> 2 \text{ and } < 4\text{mm}$	Inconclusive
$\geq 4\text{mm}$	Positive

2. The comparative cervical test (CCT)

The procedure is almost the same with SCT, except that two sites at neck are selected for PPD-B and PPD-A injection respectively.



The skin thickness at the PPD-B site and PPD-A site are measured, and the difference of skin thickness between these two measurements is calculated. The interpretation of result is based on the following table.

$\Delta B - \Delta A$ (mm)	Interpretation of result
$> 4\text{mm}$	Positive
$> 0 \text{ and } \leq 4$	Inclusive
≤ 0	Negative