ANIMAL TB
RAPID TUBERCULOSIS TEST

INTENDED USE: A two-step assay for detecting antibodies against *Mycobacterium bovis* in serum or plasma from an animal.
FOR EVALUATION ONLY! FOR RESEARCH ONLY! NOT FOR SALE!

REF 2010

FEATURES:
Ready-to-use reagents

Procedure time: appr. 20 minutes

Version: 2019-03-14

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INTRODUCTION/ FIELD OF APPLICATION

Mycobacterium bovis is the causative agent of bovine tuberculosis (TB) in animals, and it also has the potential to induce disease in humans. An unknown proportion of TB cases are caused by *M. bovis*. Zoonotic TB, caused by *M. bovis*, is present in animals in most developing countries where control measurements are not or sporadically applied, and pasteurization is rarely practiced. In countries where bovine TB is uncontrolled, most infections in human result from drinking or handling contaminated milk.

In industrialized countries, veterinary TB control and elimination programs, together with milk pasteurization, have drastically reduced the incidence of disease caused by *M. bovis* in both cattle and humans, but the control programs have not completely eliminated infection in cattle because of wild animal reservoirs.

LIONEX has developed a number of highly purified mycobacterial antigens. These antigens are being used to develop tests for sero-diagnosis of TB. Special attention is being given to cost-effective and rapid tests. The LIONEX ANIMAL TB Rapid test kits are suitable for fast and reliable detection of antibodies against mycobacterial antigens in serum or plasma samples from cattle or other animals (e.g. elephant, sheep, goat, badgers).

ADVANTAGES

- ⇨ **High sensitivity and specificity**
- ⇨ **High reproducibility**
- ⇨ **Minimal training necessary**
- ⇨ **Results in 20 min**

KIT CONTENTS

10 determinations, in-vitro-use
10 test devices
10 pipettes
1 bottles of diluent in dropper vials
1 instruction manual + 1 pattern for test result interpretation

STABILITY AND STORAGE CONDITIONS

Unopened TEST KIT: until expiry date

Opened pouch: use test cards within 1 day!

Opened diluent bottle: stable till expiry date has passed.

Unopened tests are stable at room temperature.

DO NOT FREEZE or subject to extremely high temperatures. Stability of the test kit is for 24 months from the date of manufacture. Expiry date is printed on the label.

MATERIALS NEEDED BUT NOT PROVIDED

100 µL pipette and tips (optionally).

Stop watch.

PRINCIPLE OF THE TEST

The ANIMAL TB RAPID Test is a membrane-based screening test for the rapid detection of antibodies to *M. bovis* in samples from animals. The innovative and rapid screening test is based on lateral flow immunochromatography and is among the easiest point of care assay diagnostics. The rapid test kit is suitable to test for antibodies in both serum and plasma.

After the serum or whole blood sample and diluent are put into the well on the test card using a pipette, the diluted sample passes through the gold-marked antibody binding protein (conjugate).

The conjugate attaches to the immunoglobulins contained in the sample. This antibody-conjugate complex then flows through the membrane. Two specific recombinant antigens from *M. bovis* (test line 1 and 2) and one highly purified cell wall antigen (test line 3) are immobilized on the membrane in the "T" region (test zone).

If the sample contains antibodies to one or all of these antigens, then the antibody-conjugate complex attaches itself to one or more of the test lines: one or more pink-purple bands then appears in the "T" zone of the test card. The remaining antibody-conjugate complex then passes through the card until it reaches the control zone "C". Again, a pink-purple band appears, indicating that the test has been performed properly.

LIMITATIONS

The ANIMAL TB Rapid Test Kit has been developed to detect antibodies in serum ore plasma to *M. bovis*. This test has not yet been validated for other body fluids and can yield incorrect results.

POSITIVE results, especially faint lines, must be confirmed by further diagnostic results, e.g. culture, microscopy, PCR, clinical signs.

NOTE: NEGATIVE test results do not preclude a possible TB infection or disease! **Follow the instructions and guidelines for test interpretation carefully!**

PRECAUTIONS

1. Only for in-vitro use! Do not ingest or swallow! Do not eat, drink and smoke in the laboratory! Don't work without wearing protective clothing (gloves and lab coat)!
2. All kit components should be considered as infectious agents. Wipe off serum and reagent spills with a disinfecting solution (e.g. sodium hypochlorite, 5 %)! Dispose residues of kit reagents and samples properly, e.g. by autoclaving.
3. Before use bring all reagents to room temperature (20-30 °C)!
4. Before pipetting, mix all reagents thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
5. Avoid contamination of the reagents. Close the diluent bottle immediately after use to avoid oxidation.
6. Use separate disposable pipet tips.
7. Do not use reagents from different kit lots and do not mix reagents of different kits or kit lots with one another.
8. Use all reagents within the expiry period (mentioned on the kit label). After opening, the cassettes must be used at the same day.
9. In accordance with Good Laboratory Practice (GLP) or ISO9001, all laboratory devices employed should be regularly checked for the accuracy and precision.
10. Avoid the contact of kit reagents with skin, eye or mucosa.

PREPARATION OF REAGENTS

Samples and Sample Preparation:

The serum or plasma samples **can be stored refrigerated (2 – 8 °C) for up to 48 hours.** From whole blood, the blood cells must be separated from the serum (e.g. by centrifugation) before storage.

For a longer storage keep samples at -20 °C. Avoid repeated freezing and thawing of samples. Samples appearing turbid must be clarified prior to use in assay. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

PROCEDURE

Test procedure time 20 min

1. Select the number of kits to be used. Take out the Cassette/s and lay these on a clean, flat surface.
2. Pipet 1 drop of the sample into the sample well on the card by using the pipet (provided in the kit). As alternative, you can also use 40 µL of the sample by using a micropipette.
3. Immediately add 3 drops of the diluent buffer provided.
4. Start timing and read the results after 20 min.

Strong Positives yield one or more bands at test zone "T" within 5 min. All results must be recorded after latest 25 min.

INTERPRETATION OF RESULTS

For test interpretation use the pattern attached which shows the positions of the test- and control lines on the membrane (see below).

ANIMAL TB Rapid Test

Pattern for reading the test line(s):

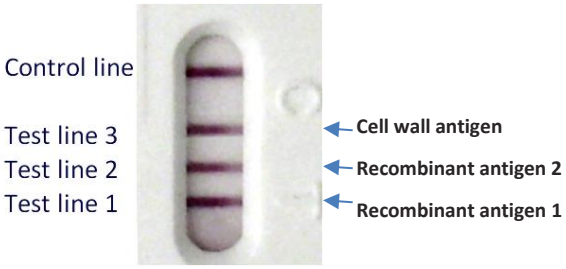
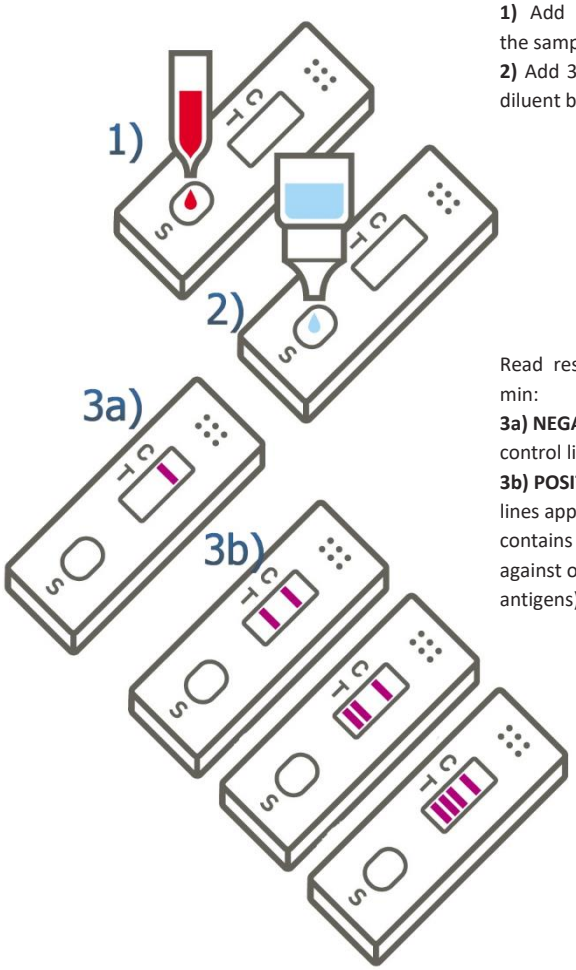


Figure 1: Rapid test procedure and possible results:

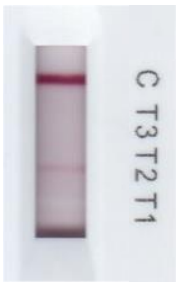


1) Add one drop of the sample.
2) Add 3 drops of the diluent buffer.

Read results after 20 min:
3a) NEGATIVE: only control line appears.
3b) POSITIVE: 1-3 test lines appears (sample contains antibodies against one of the antigens).

The test line(s) may appear distinct, weak positive or strong positive. If no control line appears, repeat the test!

NEGATIVE:



One strong line appears in control zone "C" and no test line in "T" ZONE (Figure 1, 3a). A very faint test line appears only for test line 2 (Figure 2).

Figure 2: Negative result: a very faint band appears at "T2" but no band appears in T"T3" or "T1"

POSITIVE: Up to 4 pink-purple lines appear – one appears in control zone "C" and the others in test zone "T".

If one or more test lines appear (Figure 1, 3b), the result is POSITIVE, regardless on the position of the test line ("Test line 1", "Test line 2", "Test line 3").

ATTENTION! If a very faint test line appears at "T2", and not at "T1" or "T3", the result is possibly negative!

SENSITIVITY AND SPECIFITY:

The specific antigens from *M. bovis* are evaluated for cattle. The positive animals measured were skin test positive naturally infected cows. The blood samples were taken 10 days after skin test. The sensitivity for the panel tested was 74.29 % of a specificity of more than 95 %.

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