INTERPRETATION

Calculation of the Ratio

- For each sample (S) and control, subtract the OD obtained in the well without Ag from the OD obtained in the well with Ag to obtain corrected OD.
- Calculate ratio by dividing each result by the one obtained for the positive control (P).

$$\frac{(OD_{S(Ag)} - OD_{S(\text{without Ag})})}{(OD_{P(Ag)} - OD_{P(\text{without Ag})})} = \text{RATIO}$$

Validity Criteria

The following criteria have to be met in order to validate the test:

- Negative control ratio must be less than 0.150.
- Positive control corrected OD must be greater than 0.300.

Interpretation:

- Sample ratio less than 0.250 is considered negative.
- Sample ratio greater or equal to 0.250 is considered positive.

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Mycobacterium paratuberculosis Antibody Test Kit, ELISA

Bovichek® *M. avium spp paratuberculosis* (Johne's)

Insert

2017-07-04

This is an immunoenzymatic assay for the detection of antibodies against *Mycobacterium avium spp paratuberculosis (Map)* in bovine serum.

Map is the etiological agent of paratuberculosis (synonym: Johne's disease). This disease affects several ruminant species. In infected cattle, *Map* multiplies in the intestine and induces a progressive enteritis resulting in a chronic diarrhea, wasting, drop in milk production and eventually death.

The diagnosis of Johne's is based on the demonstration of *Map* in the feces or of *Map* specific antibodies in the serum or milk. The most sensitive serological assays to detect *Map* specific antibodies are immunoenzymatic assay (ELISA). The sensitivity of these tests increases with the progression of the infection.

Some animals free from *Map* have antibodies directed against other mycobacteria that may cause non specific reactions. Pretreatment of sera with selected mycobacteria may remove these non specific antibodies.

PRINCIPLE OF THE TEST

Bovine serum samples and controls are first incubated in a buffer containing mycobacteria extracts in order to absorb non specific antibodies eventually present. Absorbed sera and controls are then incubated in wells coated with *Map* antigens (Ag) (odd columns) and in wells without Ag (even columns) that serve as negative control. The antibodies (Ab) specific to *Map* present in positive serum samples will bind the Ag in the wells. After several washes to eliminate unbound substances, a conjugate directed against bovine IgG is added. After incubation, the excess of this conjugate is eliminated by a second series of washes and its attachment is revealed with a chromogenous substrate. Following this incubation, the conjugate, if present, reacts with the substrate and a blue color develops. The reaction is then stopped and the optical densities are read. The intensity of the color is proportional to the quantity of Ab in the serum samples.

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MATERIAL

Components	Quantity
• 12 strips of 8 wells coated with *Map* Ag	2
• 12 strips of 8 wells without *Map* Ag used as control (blue sticker)	2
• Positive control	400 µL
• Negative control	400 µL
• Ready-to-use sample dilution buffer	80 mL
• Concentrated wash solution (10X)*	4 X 125 mL
• Concentrated conjugate**	400-1500 µL
• Ready-t-use substrate	60 mL
• Ready-to-use stop solution*	60 mL

* Crystals may form when these solutions are kept at 2-7°C. This will not affect the efficiency of the products. However, it is important that all the crystals are dissolved before using them. Simply bring the solutions to room temperature and agitate.
** Precise volume depends on the lot of conjugate and the recommended working dilution (see Section C)

The materials provided are sufficient for testing up to 184 samples in one test by using all the strips alternatively to form 4 microplates and distributing the appropriate controls on each microplate (see D. Test Procedure).

Material Required but not Provided:

- Purified water
- Adjustable single- and multi-channel micropipettes
- Single use micropipettes tips
- ELISA microplate washer (facultative)
- Test tubes for sample dilution
- ELISA 96-well microplate reader equipped with 650 nm filter
- Containers for preparation of solutions

PRECAUTIONS

- For *in vitro* veterinary use only.
- The materials used in this kit must be considered as infectious. Therefore, all waste must be decontaminated before being discarded.
- Do not use the kit after the expiry date indicated on the package.
- Do not mix the reagents from different serial numbers.
- The sensitivity and specificity of the test are guaranteed only if the procedures are strictly observed.
- Do not expose the substrate to either light or oxidizing agent. Always keep the substrate in a clean plastic container. This solution may cause skin or eye irritation.
- The stop solution contains an irritant and must be manipulated with caution to avoid contact with skin or mucous membranes.
- Dispose of the substrate and the stop solution according to local regulations for chemicals.
- Keep all reagents at 2-7°C and bring to room temperature before use.

EXECUTION

A. Preparation of Wash Solution

After homogenizing the concentrated wash solution (no evidence of crystals), dilute concentrated wash solution in purified water at 1/10 (e.g. 50 mL of 10 X concentrated wash solution in 450 mL of purified water). Once prepared, the solution (1X) is stable for 1 week at à 2-7°C.

B. Preparation of Controls and Samples

It is recommended to test negative and positive controls as duplicates. Mix well the controls, the samples and the dilution buffer before use. Dilute positive and negative controls as well as serum samples at 1/25 in the sample dilution buffer (e.g.: 10 µL of serum in 240 µL of dilution buffer). Make sure you use a new tip and a new test tube for each sample. Mix well and incubate at 23 ± 2°C for 30 minutes.

C. Conjugate Preparation

Dilute the conjugate with 1X wash solution (see section A) according to the dilution indicated on the Quality Control Certificate. Dilute the conjugate a few minutes prior its use and always prepare a fresh solution.

D. Test Procedure

Bring all reagents to room temperature and mix well manually before use.

1. Put strips coated with *Map* Ag on the odd columns of a frame, and strips without Ag (blue sticker) on the even columns. Put the rest of the strips in the plastic bags included for that purpose.
2. Make a schematic representation of the plate and the distribution of the controls and samples.
3. Dispense 100 µL of the absorbed positive control (see section B) into a well with Ag and a well without Ag (e.g. A1/A2).
4. Dispense 100 µL of the absorbed negative control (see section B) in the same way (e.g. B1/B2).
5. Dispense 100 µL of the absorbed samples (see section B) in the same way (e.g. C1/C2, D1/D2, etc.).
6. Incubate at 23 ± 2°C for 30 minutes.
7. Wash each well 5 times with 300 µL of 1X wash solution (see section A). Throw away all liquid contained in the plate after each wash. After the last wash, dry the plate by tapping it on absorbent paper.
8. Dispense 100 µL of the diluted conjugate (see section C) into each well.
9. Incubate at 23 ± 2°C for 30 minutes.
10. Repeat step 7.
11. Dispense 100 µL of ready-to-use substrate into each well.
12. Incubate in the dark at 23 ± 2°C for 10 minutes.
13. Dispense 100 µL of ready-to-use stop solution into each well.
14. Measure optical densities (OD) of each well at 650 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.
15. Calculate the results.