MASTAZYME™ Horse Tapeworm

Enzyme Immunoassay for the Detection of Anti-Anoplocephala perfoliata IgG Antibodies in Horse Serum

Instructions for Use

English: Pages 03–09

MASTAZYME™ Horse Tapeworm REF 713000 96 Tests

Storage: 2–8 °C
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1. Introduction

Three species of tapeworm parasites are found in European and American horses: Anoplocephala magna, Paranoplocephala mamillana and Anoplocephala perfoliata which is the most common one. Systematically they are belonging to the cestodes or flatworms. Structurally adult tapeworms all have a head called scolex and a flattened segmented body, the strobila. The head of A. perfoliata is equipped with 4 typical suckers, which is used to attach to the equine intestine. The strobila is composed of so-called proglottids. Mature proglottids at the posterior end of the ribbon-like strobila are filled with eggs. They are released and seperated from the body and are passing out with horse feces.

The reproductive cycle can roughly be described in 5 stages: 1. In an infected animal the mature proglottids with embryonated eggs become gravid and 2. are released with the faeces into the grass. 3. Here the eggs are ingested by the intermediate hosts an oribatid grass mite, in which the tapeworm develops into a larval cysteroid stadium over a 2 to 4 months period. 4. Mites in the grass habitat are swallowed by grazing horses. 5. Within the horse the cysteroids mature within four to six weeks again into the adult tapeworms.

2. Principle of the Test

MASTAZYMETM-Horse Tapeworm is an enzyme-linked immunosorbent assay for the detection of IgG(T) antibodies to Anoplocephala perfoliata in horses.

Diluted sera from horses are incubated in microwells coated with Anoplocephala perfoliata affinity purified antigens.

During incubation, any antibodies in the sample that are specific for A. perfoliata bind to the immobilised proteins. The wells are washed to remove any unbound material and affinity purified goat anti-equine IgG(T) conjugated with horseradish peroxidase, is added to the wells.

After a second incubation step, the wells are washed to remove any unbound material and the complexed enzyme is detected by the addition of chromogenic TMB substrate (3,3', 5, 5' tetramethylbenzidine). During incubation any complexed HRP enzyme will react with the substrate to produce a blueish colour. This reaction is stopped after a specific time with acid, which also causes a change in colour to an intense yellow.

The wells are then read spectrophotometrically. The intensity of the colour reaction is proportional to the amount of A. perfoliata-specific IgG(T) antibody in the sample.

3. Kit Contents

- 12 x Microtiter strips with 8 pre-coated break-apart wells coated with affinity purified 12 / 13 kDa antigen of Anoplocephala perfoliata; supplied in a strip holder, and sealed in an aluminium pouch with a sachet of silica gel.
- 4 x Self-adhesive plate sealers.
- 2 x 60 mL Sample diluent. Green coloured solution, ready to use.
- 1 x 12 mL Goat anti-equine immuno IgG (T) conjugated with horseradish peroxidase, diluted in a red coloured solution containing PBS buffer, horse serum and 0.1 % of Proclin as a preservative. Supplied ready to use.
- 1 x 1.5 mL Positive control, to be used for cut-off calculation; equine serum with antibodies specific for Anoplocephala perfoliata. Supplied ready to use.
- 1 x 1.5 mL Negative control, ready to use.
- 1 x 50 mL Washing buffer, supplied 30x concentrated, consisting of: 0.9 % (w/v) NaCl, final concentration 0.05 % (w/v) Tween 20, final concentration 0.003 % (w/v) Proclin, final concentration
4. Materials Required but not Provided

- Vortex mixer
- Precision micropipettes capable of dispensing 10 µL, 100 µL and 1000 µL
- Disposable pipette tips
- A supply of distilled or deionised water (ultrapure or HPLC grade water)
- A range of clean standard volumetric laboratory glass or plastic containers of 500 mL, 1000 mL or 2000 mL volume
- Manual washing system or an automatic positive pressure plate washer to fill and aspirate contents of wells. (N.B. plate washer relying on gravity fed washing solutions are not suitable.)
- Absorbent paper towel
- ELISA microplate reader with a 450 nm filter setting (optional: reference filter 620 nm)

5. Warning and Precautions

- The reagents supplied in this kit are for in vitro diagnostic use only
- Read instructions carefully before conducting the assay. Do not modify the instructions procedure.
- Do not use kit beyond the expiry date.
- Do not interchange reagents between different kit lots as reagents have been calibrated for each kit.
- Examine all kit reagents before performing an assay. Reagents should not be used if they appear cloudy or are suspect for any reason.
- All reagents should be stored at 2 - 8 °C and brought to room temperature before use.
- Do not re-use microwells.
- Do not mouth pipette.
- Use high quality distilled or deionised water (ultrapure or HPLC grade water) throughout.
- Do not allow microwell to dry out during the assay procedure.
- Do not heat-expose the chromogenic TMB substrate. TMB is flammable. In use avoid contact with skin, eyes and mucous membranes and keep away from heat and naked flames.
- Protect solution from exposure to direct light. The substrate incubation step should be conducted in the dark.
- The Stopping Solution contains H₂SO₄ which is corrosive. Avoid contact with skin, eyes and mucous membranes.
- Use disposable plasticware where possible. Re-usable glassware should be washed thoroughly and rinsed free of detergents before use.
• Do not cross-contaminate reagents or interchange caps on bottles. Use separate pipettes or pipette tips for each sample or reagent.

• Do not cross-contaminate specimen between wells. If while dispensing, specimen or reagent is dropped on the surface of the well strips then blot dry immediately.

• The local operating procedures for the containment of potentially infectious material have to be practised. Specimens may contain infectious organisms. Exercise extreme care at all times when handling specimen.

• Dispose of all clinical and control material safely and in accordance with local operation regulations.

6. Storage and Stability
The MASTAZYME™ Horse Tapeworm unused components can be used until the expiry date displayed on the label, if stored at 2 - 8 °C.

After opening store all reagents at 2 - 8 °C where possible. Once opened reagents are stable for at least 3 months, when not contaminated during use.

Working strength washing buffer may be stored in tightly capped containers at 15 - 30 °C for up to one month.

7. Reagent preparation
Allow all reagents, microwells and test specimens to equilibrate to room temperature before proceeding further with the assay.

Prepare a 1 in 30 dilution of the concentrated washing buffer using ultrapure water as required or dilute the entire bottle (50 mL) with 1450 mL of ultrapure water.

Dilute specimen (equine serum or plasma) 1:101 with sample diluent before testing. For example: 10 µL of sample to 1000 µL of sample diluent in a suitable tube. Mix thoroughly.
8. Assay Procedure
Carefully mix the reagents and diluted sample prior to use.

1. Prepare a sufficient number of microplate strips to accommodate the negative control, positive control and the diluted samples.
2. Pipette 100 µL of controls and samples into the appropriate wells. Cover the microwell strips with plate sealing film provided to prevent evaporation of liquid from the wells. Incubate the plate for 60 min at room temperature.
3. Discard the contents of the microwells and wash 4 times with 300 µL of washing solution. Blot dry the plates on absorbent paper.
4. Dispense 100 µL of red coloured conjugate into each well. Make a visual check to ensure all wells contain conjugate.
5. Incubate at room temperature for 60 minutes.
6. Discard the contents of the microwells and wash 4 times with 300 µL of washing solution. Blot dry the plates on absorbent paper.
7. Dispense 100 µL of ready to use TMB solution into all wells. A multichannel pipette is recommended for this step. Never use TMB solution if it has turned blue or has precipitated.
8. Cover the microwell strips with a fresh piece of plate sealing film. Incubate in the dark at room temperature for 10 ± 2 minutes.
9. After incubation terminate the reaction by adding 100 µL of stopping solution to all wells.
10. Read results after adding the stopping solution within 30 min using a suitable microplate reader with a 450 nm filter setting (reference filter 620 - 690 nm). Blank the reader against air.

9. Validation and Interpretation of Results
For batch specific control performance see attached QC document (Certificate of Kit Performance).

All OD values should be within the range of Certificate of Kit Performance.

For calculation the **OD readings should be expressed as a percentage of the positive control**, to give a percentage of positivity (PP).

Example: Recalculated result = \( \frac{1}{\text{OD pos control}} \times \text{OD sample} \times 100 \)

\[
\text{e.g. specimen: } \left( \frac{1}{1.328} \right) \times 0.850 \times 100 = 64 \%
\]

**Positive Sample:** A test sample with a **PP values greater than 60 %** indicates a positive result and is an unequivocal evidence for exposure to high tape-worm burdens.

**Negative Sample:** A test sample with a **PP values less than 30 %** are considered as negative for anti-tapeworm antibodies

**Grey zone sample:** **PP values between 30 - 60 %** describe a grey-zone area.
10. Interpretation of Results

Positive: A PP of 60 % or higher is a strong evidence for exposure to a high tapeworm burden. Such titers can be found immediately after treatment as well, because it takes 12 - 16 weeks for the antibody to decline to baseline levels.

Greyzone results: The test cannot determine whether grey-zone results are equal to current infection or just reflect past exposure to tapeworms. There is some evidence of tapeworm exposure, but it is unlikely to be related to high levels of infections.

Negative results: No indication of a current tapeworm infection.

Summary: A) PP values are proportional to infection intensity.
B) Once "high risk" horse are identified by high PP, these animals are always at risk of getting high levels of tapeworm infection and should be targeted for more intensive tapeworm treatment that other horses.

11. Limitations of Use

All test results should be interpreted in conjunction with other clinical data.

12. References


2. Proudman CJ, Trees AJ; Correlation of antigen specific IgG and IgG(T) responses with Anoplocephala perfoliata infection intensity in the horse; Parasite Immunol. 1996, 18, 499-506

3. Proudman CJ, French NP, Tress AJ; Tapeworm infection is a significant risk factor for spasmodic colic and ileal impaction colic in the horse; Equine Vet. J. 1998, 30(3) 194-199
