Version 05/2024

MegaFLUO® BABESIA canis ad us. vet.

***In vitro* diagnosticum**

Test-kit for the indirect semiquantitative immunofluorescence detection of specific IgG antibodies against *Babesia canis* in plasma or serum of the dog

INSTRUCTIONS FOR USE

DIAGNOSTIK MEGACOR
6912 Hörbranz – AUSTRIA

1. INFORMATION ON THE TEST-KIT

TEST-KIT COMPONENTS

1 Test-kit **MegaFLUO® BABESIA** canis contains:
- 10 slides coated with *Babesia canis* antigens
- 1 dropper bottle with 3.0 ml FITC anti-dog IgG conjugate
- 1 dropper bottle with 0.5 ml Positive Control
- 1 dropper bottle with 0.5 ml Negative Control
- 1 dropper bottle with 3.0 ml Mounting Medium
- 1 instructions for use

SAMPLE MATERIAL

Serum or plasma

STORAGE AND STABILITY

- The storage temperature for the whole testkit is **+2–8°C**
- The different declarations of storage temperatures on the labels of the single components refer to their storage on individual purchase (→ another expiry date).
- Shelf life: 12 months after manufacturing.

MATERIAL REQUIRED, BUT NOT PROVIDED

PBS (phosphate buffered saline) pH 7.2–7.4, washbasin for PBS, test tubes for serum dilutions, microlitre pipettes, 24×50 mm cover slips, fluorescence microscope with filter system for FITC (fluorescein isothiocyanate, excitation wavelength 465–495, barrier filter 515–555) and 400× magnification, 37°C incubator, humid chamber.

LIABILITY

The entire risk due to the performance of this product is assumed by the purchaser. The manufacturer shall not be liable for indirect, special or consequential damages of any kind resulting from the use of this product.

2. TEST PRINCIPLE

Dog sera are diluted in PBS (pH 7.2–7.4) and dropped onto the slide wells to allow an antigen-antibody reaction at 37°C in the case of a positive sample. By subsequent washing with PBS, non-bound unspecific serum proteins are washed off. In the next step, fluorescin-marked FLUO FITC anti-dog IgG conjugate is added which binds to the antigen-antibody complexes. After an incubation time of 30 minutes, non-bound conjugate is washed off with PBS. Finally, the wells are covered with Mounting Medium and cover slip. Evaluation is done with a fluorescence microscope (filter system for FITC) with 400× magnification.

3. PRECAUTIONS FOR USERS

- Make sure that the test-kit components can be correlated exactly to the particular patient.
- Use a new pipette tip for each sample or dilution.
- The conjugate is photosensitive and sensitive to heat, therefore it should be stored in the dark at 2–8°C.
- The conjugate contains Evans-blue dye, which is potentially carcinogenic. Avoid ingestion and skin contact.
- The sample material and the slides must be seen as potentially infectious and disposed of accordingly after test procedure, together with the used test-kit components.

4. IMPORTANT ADDITIONAL INFORMATION FOR TEST EVALUATION

Seroprevalence

The cut-off can vary depending on the region and the origin of the sample (dependent on the prevalence and on the state of endemicity). Therefore, it is recommended for every laboratory to determine an individual cut-off.

An acute infection (2- to 4-fold titre increase: "seroconversion") can only be determined by the titre determination of a coupled serum test (2 samples in an interval of 2–3 weeks).

The interpretation of test results should always be based on anamnestic and especially clinical data and additional laboratory parameters.

Different Babesia forms

For the coating of the slides, blood from naturally infected dogs is used. Therefore, as inclusion bodies merozoites as well as trophozoites can be seen, and they can appear in a variable number.

5. TEST PROCEDURE

1. The test-kit components (apart from conjugate!) and the sera to be tested should have room temperature at the time of application.
2. Prepare appropriate dilutions (e.g. 1:160, 1:320 and so on) with PBS for all sera to be tested. For sera found positive on a previous test it is recommended to prepare serial two-fold dilutions in PBS to determine the endpoint titre (= highest dilution that is still positive).
3. Carefully remove the slides from their foil pouch shortly before use and place them into the humid chamber. Apply 1 drop (20 µl) of the Positive and Negative Control on each slide on separate antigen wells. Pipette 20 µl of every serum dilution on separate antigen wells (fig.1). Take care that the antigen wells are completely covered.
4. Incubate for 30 minutes at 37°C.
5. Washing step: Tap remaining serum dilutions gently from the slides and shake the slides gently for 5 minutes in PBS. Repeat this step for another 5 minutes with fresh PBS. Briefly rinse the slides with distilled water. Tap remaining water gently from the slides and, if necessary, dry the teflon mask between the wells with absorbent paper or cotton bud sticks. However, do not allow the antigen wells to dry out!

   If using a washing bottle, do not focus the stream directly onto the antigen wells!
6. Place the slides back into the humid chamber and immediately drop 1 drop of FLUO FITC anti-dog IgG conjugate onto each used well (fig.2). Take care that the antigen wells are completely covered.
7. Incubate for 30 minutes at 37°C and in the dark to protect the photosensitive conjugate.
8. Repeat washing step as described in step 5.
9. Add some drops of Mounting Medium on the cover slips and place them carefully on the slides. Try to remove any possible bubbles carefully.
10. Evaluate the slides using a fluorescence microscope at 400× magnification (fig.3), comparing each well to the fluorescence pattern seen in the Positive and Negative Controls.
11. Sealed slides can be stored at 2–8°C in the dark for up to 7 days.

6. TEST EVALUATION

For the evaluation, a fluorescence microscope with a filter system for FITC (excitation wavelength 465–495, barrier filter 515–555) and 400× magnification is required.

The fluorescence pattern (form, density etc.) of the Negative and Positive Control is considered as reference pattern. Patterns of reactivity different than that seen in the Controls must be considered non-specific, which means negative!

Positive fluorescence pattern ≥ 1:160
Bright, sharp, regular stained inclusion bodies (merozoites and trophozoites) are seen in the cytoplasm (or outside in case of burst erythrocytes) of infected erythrocytes.

A further dilution of the positive samples is recommended to determine the endpoint titre (= highest dilution that is still positive).

Cut-off fluorescence pattern / recommended cut-off 1:160
The inclusion bodies show a weak (1+) yellow-green fluorescence.

Negative fluorescence pattern < 1:160
The inclusion bodies do not show any yellow-green fluorescence, they appear greyish-red or are no longer visible.

Divergent fluorescent reaction
Reaction patterns different than those seen in the Positive Control must be considered as non-specific reactions and therefore as negative.

Images are shown at www.megacor.at

EXPLANATION OF SYMBOLS

- Store at – see label
- For veterinary use only
- *In vitro* diagnosticum
- Follow instructions for use precisely
- Expiry date – see label
- LOT Lot number
- ! Do not use test-kit components from different kits, lot numbers or beyond stated expiry date.

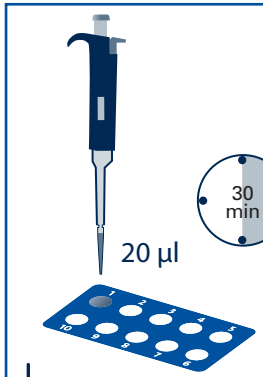

fig.1

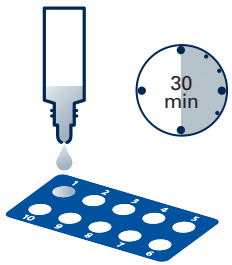

fig.2

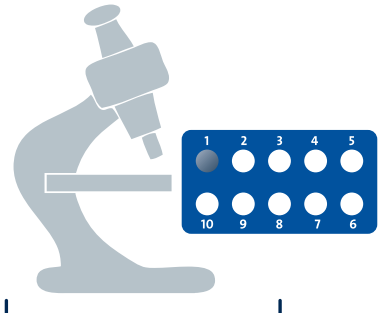

fig.3