

Manual Instruction

The Fassisi TetaCheck offers a reliable possibility to control the anti-tetanus antibody titer (antibody titer after a tetanus vaccination) in whole blood and serum of horses.

TETANUS

Tetanus infections are caused in most cases by infected wounds. The cause generally is a previous injury, the wound however must not necessarily be open. Also barely visible injuries are dangerous and may lead to an infection because of contaminants such as spores, which are mainly found in soil or street dust, usually in combination with foreign objects which get under the skin. The wound infection is caused by the toxins of the bacterium *Clostridium tetani*. The toxins get into the central nervous system through the peripheral nerves and cause spasms of the striated Muskulatur.¹ A significant rise in body temperature starts only shortly before death, which eventually occurs by respiratory paralysis. The disease is very painful for animals and almost always ends fatal.²

The pathogen occurs worldwide. An infection with the pathogen leads in unvaccinated animals in 99% of the cases to the outbreak of the disease. Depending on the individual species and the severity of the infection 55 to 90% of the diseased animals die. Unfortunately, despite today's intensive care facilities, only a very small fraction of tetanus infected horses can be rescued. Of all domestic animals, horses are the most susceptible animals for Tetanus.³ The overall mortality rate with horses is 75 to 80%.

The only effective way to prevent a tetanus infection is a preventive vaccination. Rigorous and systematic vaccination programs, beginning from the first months of life, provide the highest possible protection against a tetanus infection.

With an active control of the antibody titer in foals, the optimal time of vaccination can be narrowed easily. Too early vaccinations may eventually lead to a life-long lasting defective vaccination status.⁴

With the possibility to check the tetanus antibody titer within a few minutes, veterinarians and horse owners have the possibility to check the existing titer directly on-site to define and implement an efficient vaccination management. Severe vaccine reactions can be reduced through the improved determination of the time point of vaccination. The test is used i.a. in hospitals, to check the titer on the spot in case of severe injuries, in breeding and at sporting events.

TESTING

The Fassisi® TetaCheck is a lateral-flow sandwich-immunoassay. It functions by forming a sandwich between gold marked antibodies and antigens of the sample and immobilized antibodies in the test zone to detect pathogens.

EXPLANATION OF THE TESTING PROCESS

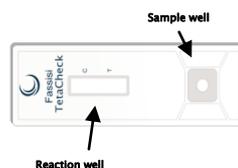
The test strips consist of different components.



When specimen is put into the sample well, it will be absorbed by the absorbing pad of the test strip. The fluid mixes with the gold labelled antigen of the conjugate pad. Due to capillary action the fluid starts to run up the test strip, crossing the test line region and afterwards the control line region. The control line always should appear to show the right functioning of the test. If the specimen contains of enough antibodies the respective test strip is testing for, a line will show in the test line region. The test lines form by building a sandwich between the gold labelled antigen from the conjugate pad, the antibody from the specimen and the immobilized antigen in the test line region. If the antibody concentration too low in the specimen the gold labelled antibody cannot connect to the immobilized antigen in the test line region and therefore no test line appears, than the test result is negative.

THE TEST CASSETTE

The test strips are located behind the plastic cover. The sample well is lying on the right side. The reaction well is located in the middle of the test cassette. The C and T mark next to the reaction well show the test and the control region.



STORAGE AND EXPIARATION

The Fassisi® TetaCheck must be storage at room temperature (2°C to 30°C).

CAUTION

- Only for veterinary and professional use
- For one-time usage only
- Use the test cassette within 10 minutes after opening the pouch
- Give no sample solution in the reaction well
- Use for each sample a new sample tube to avoid cross reactions
- Do not touch the reaction field
- Use only the original Fassisi® buffer provided in the kit
- Sample material could be infectious. Be careful with the waste disposal.
- Do not use the test after expiry date printed on the test pouch
- Do not use the test if the packing of the test cassette is damaged
- Consider the test results as invalid after the specified read-out time.

I. Contents:

- 5 test cassettes incl. pipette and drying pad
- 1 plastic bag incl. 5 sample tubes
- 1 test tube, incl. 2.5 ml reagent
- 1 instruction manual

II. Additional possible materials

- Timer

QUALITY CONTROL

In order to ensure the proper functioning of the kit, external controls are utilized as a matter of good laboratory practice. The controls should consist of a negative and positive control with minimal analyte content. It can be determined through the use of a weak positive control that a test was not negatively impacted and that the analyte can be detected with the given sensitivity of the test system.

APPLICATION & CUT-OFF OF THE TEST

The test was primarily developed for the examination of whole blood of horses. Due to its special double-antigen sandwich test setup an extended application for small animals and humans is under special conditions possible.

Cut-Off:

In the examination of horse serum, a positive test result is indicated from a tetanus antibody titre of ≥ 0.1 IU/ml. In the examination of horse full blood samples, the test line becomes visible and shows a positive test result from an antibody titre of ≥ 0.2 IU/ml.

SAMPLE PREPARATION

The sample should be tested as quickly as possible after its collection. If this is not possible, whole blood specimens can be stored at temperatures between 2°C and 8°C for a period of up to 7 hours.

The Fassisi®TetaCheck is developed for examinations of whole blood and serum.

General remarks for blood samples:

- If possible separate serum or plasma from whole blood as soon as possible to avoid haemolysis.
- Heparin or EDTA blood can be used for the plasma extraction.
- Use only clear, non-hemolyzed specimens.

Testing should be performed immediately after specimen collection. Do not expose the specimens at room temperature for a prolonged period. Serum and plasma specimens can be stored at 2-8°C for up to 3 days. For longterm storage, specimens should be kept below -20°C. Do not freeze whole blood samples!

Sample specimen needs to have room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing.

Fassisi® TetaCheck

Fassisi® TetaCheck is used to test the tetanus antibody titre in full blood and serum

E-TET-05-021

Manual Instruction

Specimens should not be frozen and thawed repeatedly. If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

TEST PROCEDURE FOR WHOLE BLOOD

- 1) Take a common blood sample from the horse to prepare the serum sample.
- 2) Open the pouch; remove the pipette and pick up the sample material with the pipette and give 2 drops of the whole blood into the sample tube. Open the buffer tube and add 4 drops of the buffer into the sample tube.
- 3) Close the sample tube and shake it well. Open the sample tube again and pick up the sample material with the pipette. Give 3 drops of sample material with the pipette into the sample well.
If the liquid is not running well after some seconds, add another drop of buffer into the sample well.
- 4) Read the results within 5-10 minutes after starting the test.

TEST PROCEDURE FOR SERUM

- 1) Take a common blood sample from the horse to prepare the serum sample. Open the pouch; remove the pipette and pick up the sample material with the pipette and give 1 drop of the serum into the sample tube.
Open the buffer tube and add 4 drops of the buffer into the sample tube.
- 2) Close the sample tube and shake it well. Open the sample tube again and pick up the sample material with the pipette. Give 2-3 drops of sample material with the pipette into the sample well.
If the liquid is not running well after some seconds, add one drop of buffer into the sample well.
- 3) Read the results within 5-10 minutes after starting the test.

TEST EVALUATION

The results of the test can be read within 5-10 minutes, after starting the test.



POSITIVE TEST RESULT:

Two red lines are shown on the membrane. The appearance of the control line indicates the correct functionality of the test. The appearance of the test line indicates a positive result. The illustrated test device on the left shows a strong test line, the antibody titer is > 1 IU / ml.



The illustrated test device on the right shows a positive test result, but with a weaker defined test line. The horse still has a sufficiently high antibody protection, but a vaccination should be done in the near future. The antibody titer in the range > 0.1 and < 1 IU / ml.



NEGATIVE TEST RESULT:

Only the control line is visible. The absence of the test line in the test region indicates a negative result.

With the cassettes on the left, an antibody titer of < 0.1 IU / ml was determined. There is no reliable protective antibody in the animal; a vaccination should be made immediately.

Attention: The test has a maximum read out time of 10 minutes after the control line appears.

INVALID:

If no line appears in the control region, the test is invalid and should be repeated.

GENERAL INFORMATION ON THE VACCINATION STATUS ⁵

Antibody Titer	Vaccination protection
$< 0,01$ IE/ml	No vaccination protection
$0,01-0,1$ IE/ml	No reliable vaccination protection (booster vaccination recommended)
$> 0,1$ IE/ml	Sufficient vaccination protection
$\geq 1,0$ IE/ml	Reliable vaccination protection

ANALYTICAL PERFORMANCE

Sensitivity and specificity of the test

Test study 2011 (Comparison study with Enzyme-linked immunosorbent assay)

Fassisi® TetaCheck	Sensitivity	Specificity
Tetanus	93,7 %	91,6 %

DISPOSAL

An accurate disposal is recommended. Sample material and test cassettes should be collected in a sealable plastic bag.

LITERATURE

- 1) Mims, C., Zuckermann, M.: Medizinische Mikrobiologie – Infektiologie mit StudentConsult-Zugang: mit Virologie, Immunologie, München, 2006
- 2) Rolle, M., Mayr, A.: Medizinische Mikrobiologie, Infektions- und Seuchenlehre, 2007, S. 504
- 3) Büthe, R.: Der Pfad der Pferde, 2011, S. 96
- 4) Knottenbelt, D. C., Holdstock, N., Madigan, J.: Neonatologie der Pferde, 2007
- 5) World Health Organization (WHO)

SYMBOLS

	Only for one use		Read user instruction carefully
	Content		Storage temperature
	Lot number		Expiry date

Rev.: 2011-07 ST

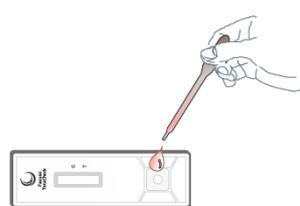
Test procedure for whole blood

Put 2 drops of whole blood into the supplied sample tube, then add 4 drops of buffer to the tube.



Close the sample tube with the lid and shake it well.

Open the tube and pick up sample fluid with the pipette. Put 2-3 drops of the sample fluid into the sample well.



After a few seconds, the liquid begins to run up the test strip.

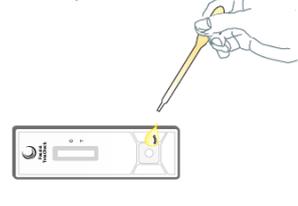
Test procedure for serum

Put 1 drop of serum into the supplied sample tube, then add 4 drops of buffer to the tube.



Close the sample tube with the lid and shake it well.

Open the tube and pick up sample fluid with the pipette. Put 2 drops of sample fluid into the sample well.



After a few seconds, the liquid begins to run up the test strip.

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Seite 2