PIGTYPE® YOPSCREEN

ELISA Test Kit to Detect Antibodies to Pathogenic Yersiniae in the Pig

Labor Diagnostik Leipzig

Instructions for Use

In vitro Diagnostic Kit for Veterinary Medicine
Registered in Accordance with §17c of the German Law on Animal Diseases
Registration No.: FLI-B 441

Applications

The PIGTYPE® YOPSCREEN is an enzyme immunoassay (ELISA) in the micro titre plate format for the detection of antibodies to pathogenic yersiniae in samples from the pig, including serum, plasma, and meat juice. Antibodies to pathogenic *Yersinia* strains, which are of concern in human medicine, will be identified.

General Information

The PIGTYPE® YOPSCREEN permits the detection of antibodies to the virulent *Yersinia* strains. The antigens (*Yersinia* Outer Proteins = Yops) used in this test are produced by pathogenic strains only, spanning all serotypes. Therefore, antibody cross reactivities to non-pathogenic species or other enterobacteria can be excluded. Additionally, the PIGTYPE® YOPSCREEN is suitable for the quantification of antibodies to yersiniae in serum or meat juice samples. It is thus an effective method of monitoring the infection status of herds over extended periods and of controlling the serological status of purchased animals.

Description of the Test Principle

The microtitre plate is coated with recombinant *Yersinia* antigens. During the sample incubation bind *Yersinia* specific antibodies to the immobilized antigens. Unbound material is removed by rinsing. The anti-pig-IgG-HRP conjugate detects antibodies bound to the antigen. Unbound conjugate is rinsed out. The colour reaction is started by adding the substrate solution and halted after 10 minutes. The optical density is measured in a spectrophotometer; the values correlate with the concentration of anti-*Yersinia*-antibodies in the sample.

Reagents

		1 plate kit	5 plate kit
1.	Test plate, contains 12 micro titre strips with 8 wells each or Test plate, micro titre plate with 96 wells, coated with recombinant, non-infectious *Yersinia* antigens	1	5
2.	Dilution Buffer, with protein and preservative, ready-to-use	60 ml	2x 125 ml
3.	Negative Control, *Yersinia*-negative pig serum, in buffer with protein stabilisers, ready-to-use	1.5 ml	1.5 ml
4.	Positive Control, *Yersinia*-reactive pig serum in buffer with protein stabilisers, ready-to-use	1.5 ml	1.5 ml
5.	Wash Buffer (10x), buffer solution with Tween and preservative	125 ml	2x 125 ml
6.	Anti-IgG-HRP, goat anti-pig-IgG peroxidase conjugate in buffer with protein stabilisers and preservative, ready-to-use	12 ml	60 ml
7.	TMB, Tetramethylbenzidine Substrate Solution, ready-to-use	12 ml	60 ml
8.	Stop Solution, 0.5 M sulfuric acid, ready-to-use (Corrosive!)	12 ml	60 ml

Additional Material and Equipment Required

Beakers, measuring cylinders, analytical pipettes, multichannel pipettes, disposable pipette tips, pipette troughs, micro titre plate spectrophotometer, tubes or plates for diluting the samples, distilled water

Precautions and Warnings

Store the reagents at 2-8 °C and only bring them to room temperature (18-25 °C) immediately before use. In case of salt crystallisation in the 10x Wash Buffer dissolve the salt crystals by mixing and careful warming. Wash Buffer (10x, bottle 5) and Stop Solution (bottle 8) may be stored at room temperature (18-25°C) to avoid salt crystallisation. Store the remaining test strips in the re-sealed pack with desiccant at 2-8 °C until next use. The test strips can be stored at least for 6 weeks after opening the plate pack.

The test should only be performed by persons qualified for laboratory work. Store the TMB Substrate Solution in the dark and do not expose it to intense light or to sunlight during the test. The components of the test kit may not be contaminated or mixed with components from other batches. Do not use the components of the test kit past expiration date. The water used for diluting the buffer concentrate, particularly water from ion-exchange plants, may interfere with the reaction if it is not pure enough. Water of the quality of double distilled water or highly purified water (Milli-Q) is suitable.

To guarantee the precision of the results, it is absolutely essential to follow the usual precautions for ELISA procedures, including the use of clean glass devices, careful pipetting and rinsing during the test, and strict adherence to the indicated incubation times. The kit contains hazardous substances (Sulphuric acid). All sample residues and objects which have come into contact with samples must be decontaminated or disposed of as potentially infective material.

Preparation of the Reagents and Samples

Wash Buffer:

Wash Buffer (10x), bottle 5, dilute 1:10 with distilled water,
e.g., for one test plate dilute 50 ml Wash Buffer (10x) in 450 ml distilled water and mix.

Serum and plasma:

Before using the serum or plasma samples in the assay, dilute them 1:100 with Dilution Buffer, e.g. 5 µl serum or plasma are diluted in 495 µl Dilution Buffer and mixed. Be sure to change pipette tips for each sample. Controls are ready-to-use, do not dilute them.

Meat juice:

Freeze approx. 10 g blood and fat free muscle meat, e.g. from the diaphragm column, in the SALMOSTORE® meat juice container and then thaw it. In the monitoring programmes, the meat sampling sites on the animal body can be laid down exactly. The frozen meat samples can be stored for several months at -20 °C before testing. The meat juice released from the thawed meat collects in the tube of the meat juice container.

Before using the meat juice samples in the test, dilute them 1:10 with Dilution Buffer, e.g. 25 µl are diluted in 225 µl Dilution Buffer and mixed.

Test Procedure

Bring all reagents to room temperature (18-25 °C) before use and mix well.

1. Record the positions of the controls (in duplicates) and samples on the Test Plate in a test protocol, e.g. Negative Control = A1/B1; Positive Control = C1/D1; other positions of the samples.
2. Filling the Test Plate: Pipette 100 µl of each of the ready-to-use Negative and Positive Control (in duplicates), the 1:100 diluted serum or plasma samples, and/or the 1:10 diluted meat juice samples into the Test Plate wells. Cover the Test Plate.
3. Incubate for 60 min at room temperature and then empty the wells by aspiration or effusing.
4. Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.
5. Add 100 µl ready-to-use anti-IgG-HRP to each well. Cover the Test Plate.
6. Incubate for 30 min at room temperature and then empty the wells by aspiration or effusing.
7. Rinse each well 3x with 300 µl of prepared Wash Buffer. Remove the buffer after each rinse.
8. Add 100 µl TMB Substrate Solution to each well.
9. Incubate for 10 min at room temperature in the dark.
10. Stop the reaction by adding 100 µl of Stop Solution per well.
11. Calibrate the spectrophotometer against air as blank. Measure the optical density (OD) in the spectrophotometer at 450 nm immediately or within 20 min of stopping the reaction. Measuring at a reference wavelength (620-650 nm) is optional.

Test Validation

For the assay to be valid the measured OD for the Positive Control must be ≥ 1.3; the measured OD for the Negative Control must be ≤ 0.15.

Calculation

1. Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

2. Subtract the mean OD of NC from the OD of the sample and from the OD of the PC.

3. The sample activity value is calculated from the ratio of the OD of the sample to the mean OD of the PC according to the following formula:

$$\text{Activity (OD \%)} = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}} \times 100$$

Evaluation

Samples with activity values < 10 OD % are negative. Specific antibodies to pathogenic yersiniae could not be detected.

Samples with activity values ≥ 10 OD % and < 20 OD % are doubtful. Doubtful results should be grouped to the majority of the positive or negative results. It is recommended to re-test doubtful results after a few weeks.

Samples with activity values ≥ 20 OD% are positive. Specific antibodies to pathogenic yersiniae were detected.

To classify the populations in specific monitoring programmes, other cut-off values for positive samples may be laid down.

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