Introduction

Trichinellosis caused by the Nematode Trichinella is a zoonotic disease which occurs worldwide and affects a broad range of different species including mammals, birds and amphibians. Currently 11 different subspecies (8 designated as species) have been recognized in this genus. The species that are of main importance in Europe are Trichinella spiralis, Trichinella britovi, Trichinella pseudospiralis, and Trichinella nativa. Trichinella spiralis is found in production animals (pigs, horses in temperate climate zones and can also be found in animals in close contact with these production animals (e.g. dogs, cats, rats). Trichinella britovi is mainly found in wildlife. Trichinella pseudospiralis is distributed worldwide and is also found in birds. Trichinella infections of pigs are of major concern since humans can be infected by eating raw or insufficiently cooked meat.

The PrioCHECK® Trichinella Ab is a reliable and fast diagnostic test for detection of antibodies against Trichinella in porcine serum and meat juice samples and can be used for monitoring and surveillance purposes.

Test Principle

E/S antigen as the major antigenic protein complex is coated on the ELISA plate. Serum or meat juice samples are incubated on the plate. A peroxidase (POD) labeled anti-pig antibody is used for the detection of antibodies bound to the E/S antigen. Color development using TMB substrate measured optically at a wavelength of 450 nm shows the presence of antibodies directed against Trichinella spp. The PrioCHECK® Trichinella Ab follows a four step protocol, consisting of Sample Preparation, Sample Incubation, Conjugate Incubation, and Detection. One plate with 90 prepared samples can be analyzed within 90 minutes.

Kit Components

Store kit at 5±3°C until expiry date. See kit label for actual expiry date. The shelf life of diluted, opened or reconstituted components is noted below, where appropriate. Chemical hazard data are available in section “Safety Regulations and R&S Statements” (Appendix IV).

Component 1 Test Plate (Strip Plate) Five Test Plates are delivered in vacuum bags containing a desiccant bag.

Component 2 Sample Diluent (Ready-to-use) Two bottles containing 60 ml of Sample Diluent. The Sample Diluent is used to dilute the samples. Color of solution: yellow

Component 3 Washing Fluid (20x) (20x concentrate, dilute before use). Two bottles containing 60 ml Washing Fluid (20 x). Prepare Washing Fluid working solution by mixing 1 part Washing Fluid (20 x) with 19 parts demineralized water or water of equal quality and 0.2 parts of Washing Fluid Additive (equals 1%). Mix until a clear solution is obtained.

Remark: If the Washing Fluid (20x) shows a precipitate, warm the bottle in a 30°C water bath until the precipitate is completely redissolved. See Appendix II

Stability of Wash Fluid working solution: 2 weeks at 22±3°C.

Component 4 Washing Fluid Additive One bottle contains 30 ml Washing Fluid Additive. 1% Washing Fluid Additive has to be added to the diluted washing fluid. Mix until a clear solution is obtained.

Component 5 Conjugate Diluent (Ready-to-use) One bottle contains 60 ml Conjugate Diluent. The Conjugate Diluent is used to dilute the Conjugate. Color of solution: red

Component 6 Conjugate (30x) (30x concentrate, dilute before use). One bottle containing 2 ml Conjugate. Prepare 1x Conjugate by mixing 1 part of Conjugate with 29 parts of Conjugate Diluent. Dilute the amount of Conjugate necessary to run the test, just prior to use.

See Appendix II

Additional Kit Contents:

Package Insert

Component 7 Chromogen (TMB) substrate (Ready-to-use) One bottle containing 60 ml Chromogen (TMB) substrate. The Chromogen (TMB) substrate is the substrate for the color reaction.

Component 8 Stop Solution (Ready-to-use) One bottle containing 90 ml Stop Solution. The Stop Solution is used to stop the color development.

Component 9 Deionized Water One vial containing 10 ml Deionized Water. Deionized Water is used to reconstitute the lyophilized control samples.

Component 10 Positive Control (Lyophilized) One vial containing lyophilized Positive Control. The Positive Control is reconstituted by adding 150 µl of Deionized Water (supplied with the kit). Mix by vortexing thoroughly and inverting the vial several times. Store at -20 to -80°C after reconstitution. Can be aliquoted or may be frees-thawed up to 5 times.

Component 11 Weak Positive Control (Lyophilized) One vial containing lyophilized Weak Positive Control. The Weak Positive Control is reconstituted by adding 150 µl of Deionized Water (supplied with the kit). Mix by vortexing thoroughly and inverting the vial several times. Store at -20 to -80°C after reconstitution. Can be aliquoted or may be frees-thawed up to 5 times.

Component 12 Negative Control (Lyophilized) One vial containing lyophilized Negative Control. The Negative Control Sample is reconstituted by adding 150 µl of Deionized Water (supplied with the kit). Mix by vortexing thoroughly and inverting the vial several times. Store at -20 to -80°C after reconstitution. Can be aliquoted or may be frees-thawed up to 5 times.

Additional Material Required

General: Laboratory equipment according to national safety regulations.

- Demineralized or water of equal quality must be used
- Dummy plate, used for sample dilution (e.g. clear colorless round bottom 96 well plates) or equivalent
- Single channel pipette (10 – 100 µl)
- Single channel pipette (20 -200 µl)
- Multichannel pipette (5 – 50 µl)
- Multichannel pipette (50 – 300 µl)
- Pipette tips (as recommended by pipette manufacturer)
- Solution reservoirs
- Vortex

Sample preparation:

- Dedicated blood collection tubes

The diagnostic assay for the detection of antibodies directed against Trichinella spp. in serum and meat juice samples of pigs is based on ELISA technology.
PrioCHECK® Trichinella Ab

- Dedicated meat juice collection tubes

Analysis of Results:
Plate Reader, e.g. Tecan Sunrise or equivalent. The reader has to have an appropriate filter set to read the plates at 450 nm.

Optional:
Plate washer e.g. Tecan EIA Tray Washer or equivalent.

Test Procedure

Precautions
National guidelines for working with animal samples must be strictly followed. The PrioCHECK® Trichinella Ab must be performed in laboratories suited for this purpose. Samples should be considered as potentially infectious and all items which contact the samples as potentially contaminated.

Chemical hazard data are available in section “Safety Regulations and R&S Statements” (Appendix IV).

Notes
To achieve optimal results with the PrioCHECK® Trichinella Ab, the following aspects must be considered:
- The Test Procedure protocol must be strictly followed.
- Pipette tips have to be changed for every pipetting step.
- Separate solution reservoirs must be used for each reagent.
- Kit components must not be used after their expiry date or if changes in their appearance are observed.
- Kit components of different kit lot numbers must not be used together.
- Demineralized water or equal must be used for the test.

SAMPLE PREPARATION

- Serum can be obtained using standard methods.
- If meat juice is tested, a piece of muscle tissue (e.g. 10 g (preferable tongue, diaphragm or masseter) is either freeze-thawed in a dedicated device or alternatively the piece of meat can be squeezed to obtain meat juice.

SAMPLE DILUTION:

Preparatory Steps
- Reconstitute the control sample by adding 150 μl of Demineralized Water (delivered with the kit).
- Mix by vortexing thoroughly and inverting the vial several times or use already reconstituted control samples that have been stored at -20°C to -80°C

Sample dilution for serum samples
- Use a Dummy Plate or equivalent for first sample dilution
- Add 10 μl of Positive Control to wells A1 and B1 of the Dummy Plate.
- Add 10 μl of Weak Positive Control to wells C1 and D1 of the Dummy Plate.
- Add 10 μl of Negative Control to wells E1 and F1 of the Dummy Plate.
- Add 10 μl of serum samples to the remaining wells of the Dummy Plate.
- Add 90 μl of Sample Diluent to wells A1 to F1 (control samples) of the Dummy Plate.
- Add 100 μl of meat juice sample to the remaining wells of the Dummy Plate.
- Provide 80 μl of Sample Diluent to each well of the Test Plate.
- Transfer 20 μl of the samples and diluted controls from the Dummy Plate to the Test Plate.

SAMPLE INCUBATION

- Incubate the samples on the Test Plate for 30±1 minutes at room temperature (22±3°C).
- Wash the Test Plate four times with 300 μl of Wash Fluid working solution (see Appendix II).
- Add 100 μl of the diluted Conjugate to each well of the Test Plate.
- Incubate the Test Plate for 30±1 minutes at 22±3°C.
- Wash the Test Plate four times with 300 μl of Wash Fluid working solution (see Appendix II).

DETECTION

Substrate reaction
- Add 100 μl of the Chromogen (TMB) substrate to each well on the Test Plate.
- Incubate the Test Plate for 15±1 minutes at 22±3°C.
- Add 100 μl of the Stop Solution to each well of the Test Plate.
- Remark: The addition of stop solution 15±1 minutes after the first well was filled with Chromogen (TMB) Substrate solution. Add the Stop Solution in the same order as the Chromogen (TMB) Substrate solution was dispensed.

Detection
- Shake the Test Plate shortly (5-10 s.) either on an orbital shaker (~300 rpm) or manually on the working bench.
- Read the Test Plate in the ELISA reader at 450 nm within 15 minutes.
- Recommendation: Use a reference filter at 620 nm.

RESULT INTERPRETATION

Calculation of results

\[
\text{OD}_{\text{450 max}} = \text{OD}_{\text{450 max}} \times 100 = \% \text{ positivity}
\]

Validation criteria
- The mean OD_{450} of the Positive Controls must be >1.0
- The mean percentage of positivity (PP) of the Weak Positive Controls must be >35%
- The mean OD_{450} of the Negative Controls must be <0.2

If these criteria are not met, the results are invalid and the samples have to be retested.

Interpretation of results
Results obtained above or equal the cut-off of 15 PP are considered positive.
Results obtained below the cut-off of 15 PP are negative.

Appendix I

Notice
This manual is believed to be complete and accurate at the time of publication. In no event shall Prionics AG be liable for incidental or consequential damages in connection with or arising from the use of this manual.

Liability
Prionics AG warrants its products will meet their applicable published specification when used in accordance with their applicable instructions and within the declared products life time. Prionics AG makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose. The warranty provided herein and the data, specifications and descriptions of Prionics AG products appearing in Prionics AG published catalogues and product literature may not be altered except by express written agreement signed by an officer of Prionics AG. Representation, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

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Prionics AG shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by any customer from the use of its products.

Prionics AG is an ISO 9001:2000 certified company.

Appendix II

Preparation of Washing Fluid working solution and Conjugate Solution

Washing Fluid working solution
Mix indicated volumes of demineralized water and Washing Fluid (20x). Add 1% of Washing Fluid Additive to the solution and mix properly to obtain the desired volume of Washing Fluid working solution.
- Transfer 50 ml Washing fluid (20x) to a 2 l bottle.
- Add 940 ml of demineralized water and mix.
- Add 10 ml of Washing Fluid Additive, mix properly until a clear solution is obtained (approx. 30 minutes).

Conjugate working solution
Mix indicated volumes of Conjugate 30x with the appropriate amount of Conjugate Diluent (supplied with the kit) to obtain the desired amount of Conjugate.

<table>
<thead>
<tr>
<th>Volume Washing Fluid working solution</th>
<th>amount of Washing Fluid working solution</th>
<th>amount of D1</th>
<th>amount of Washing Fluid working solution</th>
<th>amount of Washing Fluid working solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 l</td>
<td>50 ml</td>
<td>940 ml</td>
<td>5 ml</td>
<td>20 ml</td>
</tr>
<tr>
<td>0.5 l</td>
<td>25 ml</td>
<td>470 ml</td>
<td>5 ml</td>
<td>20 ml</td>
</tr>
<tr>
<td>0.3 l</td>
<td>15 ml</td>
<td>282 ml</td>
<td>3.0 ml</td>
<td>20 ml</td>
</tr>
</tbody>
</table>

Remark: If the Washing Fluid (20x) shows precipitations, warm the bottle in warm water bath (approximately 30°C) until all salts are completely redissolved.
Appendix III

Pipetting Schemes

Recommended pipetting scheme for Dummy Plate and Test Plate.

<table>
<thead>
<tr>
<th>Component 1</th>
<th>Component 2</th>
<th>Component 3</th>
<th>Component 4</th>
<th>Component 5</th>
<th>Component 6</th>
<th>Component 7</th>
<th>Component 8</th>
<th>Component 9</th>
<th>Component 10</th>
<th>Component 11</th>
<th>Component 12</th>
</tr>
</thead>
</table>

Appendix IV

Safety Regulations and R&S Statements

National Safety Regulations must be strictly followed.

R&S Statements

Component 1
Test Plate
Hazard Code: This product is not classified according to EU regulations.

Component 2
Sample Diluent (Ready-to-use)
Hazard code: Xi Sensitising
R43: May cause sensitization by skin contact.
S24: Avoid contact with skin.
S37: Wear suitable gloves.

Component 3
Washing Fluid (20 x conc.)
Hazard code: Xi Sensitising
R43: May cause sensitization by skin contact.
S24: Avoid contact with skin.
S37: Wear suitable gloves.

Component 4
Washing Fluid Additive
Hazard Code: This product is not classified according to EU regulations.

Component 5
Conjugate Diluent
Hazard Code: This product is not classified according to EU regulations.

Component 6
Conjugate 30x
Hazard Code: This product is not classified according to EU regulations.

Component 7
Chromogen (TMB) Substrate (Ready-to-use)
Hazard Code: This product is not classified according to EU regulations.

Component 8
Stop Solution
Hazard Code: R35 Causes severe burns.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Component 10
Positive Control (Lyophilized)
Hazard code: The product does not have to be labelled due to the calculation procedure of the “General Classification guideline for preparations of the EU” in the latest valid version.

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