SERLOGICAL DETECTION OF SPECIFIC ANTIBODIES TO
TRICHINELLA SPIRALIS BY ELISA
IN SERA AND MEAT JUICE

192 Reactions

POURQUIER® ELISA Trichinella Serum Screening
VERSION: P08500/03 - 09-08-2007
**INTRODUCTION**

Trichinellosis is a zoonosis that is most often due to the nematode *Trichinella spiralis*. Sometimes other species can be responsible for this affliction: mainly *Trichinella pseudospiralis*, *Trichinella britovi* and *Trichinella nativa*. Its prognosis is almost always benign in animals, but it is always severe in man. Classically, humans are contaminated by ingestion of insufficiently cooked pork meat, but half of the cases reported in the European Union over the last twenty years are due to horse meat imported from Eastern European countries (Poland, Rumania, and former Yugoslavia) or North America (United States, Canada, and Mexico). The other human cases come from either pig farming in the open or (industrial farming is virtually *Trichinella* free in the EU) from wild boars (Pozio, 1998). Over a considerable time the systematic tracking at slaughtering has been based on the direct detection of the larvae, either by trichinoscopy or by muscle digestion. In the absence of alternate superior methods, serology, based on the excretory / secretory (E/S) antigen, remains an epidemiological tool and though it is not suitable as a method to monitor disease control.

The animals, which are infected by *Trichinella spiralis*, develop immunological reactions during the infection and the migration of the larvae from the intestine to the muscles. Seropositive animals must be considered to be infected. The proposed test allows the detection of antibodies directed to *Trichinella spiralis* (as well as to *T. pseudospiralis*, *T. britovi* and *T. nativa*) in serum and meat juice of swine, wild boars and horses.

**PRINCIPLE OF THE TEST**

1. The *Trichinella* E/S antigen is supplied coated to the wells of the polystyrene microplate.
2. The samples are diluted and incubated in the wells of the microplate. If antibodies specific to *Trichinella spiralis* are present in the sample, they form antigen-antibody complexes by which the antibodies are fixed to the microplate wells.
3. After washing, a protein G coupled to the horse radish peroxidase, is added in the wells and incubated. This conjugate binds to the immune-complex.
4. After washing, the enzyme substrate (TMB) is added to the wells. If the serum is positive, the enzyme transforms the substrate into a blue compound becoming yellow after blocking. The intensity of the colour is a measure of the level of antibodies in the sample.

**DETECTABILITY**

The analysis of pork sera coming from *Trichinella* free zones has shown that the S/P% values were most often around 10% and never higher than 40%. As a result the cut-off has been fixed at 40% including a doubtful zone between 30% and 40%. The cut-off is set by using a positive control sample supplied with the kit, which must be added to each plate.

**PRECAUTIONS FOR USE**

1. Do not place the pipette in the mouth when testing reagents.
2. Avoid contact of the substrate (TMB*) with skin, mucous membranes and eyes.
3. *(Stop-solution)* containing H₂SO₄*(0,5M) acid, could cause serious burns in case of contact with skin, mucous membranes and eyes.
4. Even if the material in the package does not contain any contaminating element, and that the sera are, in theory, non - infectious, it is nevertheless advised to decontaminate the whole disposable elements, either by immersion for at least 1 hour in freshly prepared 5% sodium hypochlorite, or by autoclaving them at 120°C for a minimum of 1 hour by any other method in accordance with the reglementation in force, before discarding.
5. Controls contain sodium azide* and may be toxic if ingested.

* The product’s toxicity form is available at the Institut Pourquier.

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# Kit Contents and Storage of Reagents

It is recommended to bring at room temperature (21°C ± 5°C) all the reagents of the kit at least one hour before use (except the conjugate).

<table>
<thead>
<tr>
<th>COMPONENTS</th>
<th>QUANTITY</th>
<th>STORAGE AND NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monowell Coated microplates</td>
<td>2</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If a microplate is not entirely used, it may be stored for later use during 3 months if it is immediately closed in an airtight container and stored at +5°C (± 3°C).</td>
</tr>
<tr>
<td>Concentrated (x 20) wash solution</td>
<td>1 x 100 ml bottle</td>
<td>+5°C (± 3°C)</td>
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<tr>
<td></td>
<td></td>
<td>• May form crystals at +5°C (± 3°C), but these rapidly disappear at +21°C (± 5°C). A gentle agitation of the solution will accelerate the dissolution of the crystals</td>
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<tr>
<td></td>
<td></td>
<td>• This solution can also be stored at +21°C (± 5°C) for 1 month, if the vials are well closed, in order to be immediately ready to use when needed.</td>
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<tr>
<td></td>
<td></td>
<td>• The &quot;Concentrated (X 20) wash solution&quot; is the same for all the kits of INSTITUT POURQUIER and can be used equally in the different kits.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• After dilution, the Wash Solution can be stored for 3 days at +5°C (± 3°C).</td>
</tr>
<tr>
<td>Dilution Buffer 2 green</td>
<td>1 x 120 ml bottles</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Dilution Buffer 1 blue</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Positive control (freeze-dried)</td>
<td>1 x 0,5 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Negative Control (freeze-dried)</td>
<td>1 x 0,5 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• For the reconstitution, dispense the distilled water and wait for 10 min before shaking in order to homogenize the solutions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• After reconstitution, the positive and the negative control sera must be stored in aliquots at a temperature ≤ -16°C. They can be frozen and unfrozen up to five times without any loss of activity. A storage at +5°C (± 3°C) will lead to a significant increase of the background noises.</td>
</tr>
<tr>
<td>Conjugate: Protein G / peroxidase</td>
<td>1 x 0,3 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• The diluted conjugate solution cannot be stored</td>
</tr>
<tr>
<td>Revelation solution 3</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td>Ready to use (TMB)</td>
<td></td>
<td>• This solution can be slightly bluish at +5°C (± 3°C) and becomes colourless at +21°C (± 5°C).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Thus it can be left on the draining board at +21°C (± 5°C) up to 1 week (if the vial is well closed in an airtight way), in order to be immediately available when needed.</td>
</tr>
<tr>
<td>Stop Solution (H₂SO₄; 0.5M solution)</td>
<td>1 x 120 ml bottle</td>
<td>+5°C (± 3°C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• It can be stored at +21°C (± 5°C) up to 1 month (if the vial is well closed), in order to be immediately available when needed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• It is the same for all the kits of INSTITUT POURQUIER and can be used equally in the different kits.</td>
</tr>
</tbody>
</table>

Using Instructions
MATERIAL REQUIRED BUT NOT INCLUDED IN THE KIT

1) Microplate reader
2) Centrifuge
3) Centrifuge tubes
4) Vortex or similar type agitator
5) Microplate washing system that distributes 300 µl per well
6) Precision Micropipettes and Multi-dispensing micropipettes (the precision required must be lower or equivalent to 10% for volumes lower or equivalent to 10 µl and 5% for the other indicated volumes)
7) Disposable pipette tips
8) Distilled water: the water used for the reconstitution of the «Concentrated (20X) wash solution » may be produced by a conventional distillation system or any other high-performance water purification system (reverse osmosis, purification on resin and activated charcoal ...).
9) Microplate covers (aluminium or adhesive foil).

INSTRUCTIONS FOR USE

1) DEPOSITING THE SERA

a) Preparation of the controls
Reconstitute the positive and negative controls with 0.5 ml of distilled water.

b) Treatment of samples

• Serum samples
The controls and sera to be tested are diluted to 1/20 and incubated for 30 min (± 5 min.) at 21°C (± 3°C) by using the following method (see note 2 and 3):

⇒ Dispense:
  • 190 µl of "Dilution buffer 2 » per well
  • 10 µl of diluted negative control in A1
  • 10 µl of diluted positive control in B1 and C1
  • 10 µl of each serum to test per well (only one well per sample to test)
⇒ Homogenize the contents of the wells by gently shaking the plate (see note 1)
⇒ Cover the plate (with a lid, aluminium or adhesive foil) and incubate for 30 min (± 5 min) at +21°C (±5°C).

• Meat juice samples
Muscle juice samples to be tested are diluted to 1/2 and incubated for 30 min (± 5 min.) at 21°C (± 3°C) by using the following method (see note 2 and 3):

⇒ Dispense:
  • 50 µl of "Dilution buffer 2 » per well
  • 50 µl of muscle juice to test per well (only one well per sample to test)
⇒ Homogenize the contents of the wells by gently shaking the plate (see note 1)
⇒ Cover the plate (with a lid, aluminium or adhesive foil) and incubate for 30 min (± 5 min) at +21°C (±5°C).
| A  | N                | N = Negative control |
| B  | P                | P = Positive control  |
| C  | P                | 1 = Sample to test n° 1 |
| D  | 1                | 2 = Sample to test n° 2 |
| E  | 2                | 3 = ......................... |
| F  | 3                |                           |
| G  | 4                |                           |
| H  | ..               |                           |

**Figure 1**: Distribution of the Samples

**Note:**
1. The individual filling of the 96 wells is sometimes a long process. In order to standardize the sample incubation time, the controls and samples are prepared in plates with 96 wells with U-shaped-bottoms. It is therefore possible to transfer them rapidly (column by column) by using a multi-dispensing pipette. It is nevertheless essential to dilute the samples in the same way as the controls.
2. The position of the controls in A1, B1 and C1 is not very important. They may be dispensed anywhere on the plate.
3. Laboratories, which use automatic methods or which prefer pre-diluting the samples in order to improve the reproducibility, may not have enough reagents (i.e. "concentrated X 20 wash solution" or "Dilution Buffer "...). Extra reagents can be supplied free of charge, on request.

2) **WASHING**

a) Dilute a vial of "concentrated (X 20) wash solution" in 1900 ml of distilled water. This solution is hereafter called "wash solution". The dilution can be carried out before the elimination of crystals appeared at +5°C (±3°C), so long as the whole 100 ml vial is used.

b) Empty the content of the plate by «flick-off» or better by a half-automatic or automatic method.

c) Fill all the wells of the plate with the wash solution; then empty them again.

d) Repeat the step c) twice (a total of 3 washes).

**Note:**
When a lot of plates are processed at the same time, it is possible (in order to synchronize all the steps) to leave the plates full of "Wash solution" during one hour without modifying the validity of the test.

3) **DEPOSITING THE CONJUGATE**

a) Dilute the conjugate to 1/100 with the "Dilution buffer 1"

b) Dispense 100 µl per well

c) Cover the plate (with a lid, aluminium foil or adhesive) and leave to incubate for 30 min (± 3 min.) at 21°C (± 3°C)

4) **WASHING**

a) Empty the content of the plate by «flick-off » or by another half-automatic or automatic method.

b) Fill all the wells on the plate with the wash solution; then empty them again.

c) Repeat the step b) twice (total of 3 washes)
5) REVELATION

a) Dispense 100 µl of «Revelation solution N° 3» per well.
b) Incubate the plate at +21°C (± 5°C) for 10 minutes (away from the light).
c) Dispense 100 µl of “stop solution” per well.
d) Shake gently the plate until the coloured solution is homogenized. Wipe carefully the underside of the plate.

Note:
The 10-minute revelation period as outlined above gives the O.D. values provided in the paragraph "INTERPRETATION", when implemented in our laboratories. Nevertheless the rate of colour revelation can be slightly modified by different factors (quality of the washes, quality of water used, precision of the pipetting, temperature of the reaction...). Depending then on these factors, the revelation step may give O.D. values higher or lower than those expected. In this case, the reaction may be stopped at 10 min ± 5 min. The reading can be done up to 1 hour after having stopped the reaction, if the plates are kept in the dark.

6) READING

Read the optical densities at 450 nm (OD.₄₅₀). The microplate reader must be blanked on air.

VALIDATION CRITERIA

The results can be considered reliable if:

♦ The positive control has a minimal OD₄₅₀ mean value of 0.350 and
♦ The ratio between the OD₄₅₀ value of the positive control and OD₄₅₀ value of the negative control is greater than or equal to 3.5.

INTERPRETATION

Calculate for each sample, the S/P percentage:

\[
\%S/P = \frac{OD_{450} \text{ value of the sample}}{OD_{450} \text{ value of the positive control}} \times 100
\]

- Any sample with a S/P equal or lower than 30% is considered as negative.
- Any sample with a S/P is between 30% and 40% is considered to be doubtful.
- Any sample with a S/P equal or higher than 40% is considered to be positive.
For wild boars:
- Any sample with a S/P equal or lower than 90% is considered as negative.
- Any sample with a S/P is between 90% and 100% is considered to be doubtful.
- Any sample with a S/P equal or higher than 100% is considered to be positive.

Note:
Animals showing a doubtful result should be analysed once again on a new samples. If possible the second analysis should be programmed at least two weeks after the first one.

BIBLIOGRAPHY

3. Directive 92/117 CEE
4. Directive 77/96 CEE

LEGEND

☞ : Modification in the using instructions

! : Minor modification in the using instructions