Diagnostic Sensitivity and Specificity using Crocodile miniWorkstation

Diagnostic Sensitivity and Specificity of samples tested with the Crocodile miniWorkstation and PrioCHECK® Toxoplasma Ab porcine ELISA from Prionics AG

Introduction:

An ELISA protocol contains typical routine steps such as the addition of different reagents, incubations, microplate washing steps and OD-measurements. Laboratory benches are often cluttered by large instruments or multiple instruments required for assay procedure. Lack of space negatively affects productivity. The new Crocodile miniWorkstation combines the functionality of five individual instruments in a footprint the size of a standard stand-alone ELISA reader. This note will demonstrate the diagnostic sensitivity and specificity of the system using the ELISA test PrioCHECK® Toxoplasma Ab porcine (Prionics AG).

Toxoplasmosis is caused by the protozoan parasite Toxoplasma gondii, which belongs to the family of Sarcocystidae. Toxoplasma infections are widespread in humans and many other species of warm-blooded animals. Occurrence is world wide, however, the prevalence in human and animal populations varies greatly among countries.

Diagnostic solution and Performance with PrioCHECK® Toxoplasma Ab porcine

The PrioCHECK® Toxoplasma Ab porcine is a reliable and efficient diagnostic test for the detection of antibodies against Toxoplasma gondii in porcine serum, plasma and meat juice samples and can be used for monitoring and surveillance purposes. The PrioCHECK® Toxoplasma Ab porcine showed a sensitivity of 98% and specificity of 99.6% in an evaluation on 50 positive and 270 negative porcine serum samples. With meat juice samples (33 positive and 116 negative) the sensitivity and specificity were 97% and 100%, respectively. The status of all samples was confirmed by IFAT, WB and ELISA in national reference laboratories (www.prionics.com).

Materials:

Instrumentation: Crocodile miniWorkstation
Single channel pipette (20-200 µl)

Reagents: PrioCHECK® Toxoplasma Ab porcine. Product N.: 7610230; Lot TX100401M; exp Date April 30th 2011
Demineralized water

Consumables Solution reservoirs
Pipette tips
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**Method:**

Test procedure

Diagnostic sensitivity is defined as the ability to correctly identify infected animals whereas diagnostic specificity is defined as the ability to correctly identify non-infected animals.

To determine diagnostic sensitivity and specificity 90 samples were analysed using the **Crocodile** miniWorkstation. 20 of the used samples are confirmed positive and 70 samples are confirmed negative samples. Reagent and Sample dilution was performed as described in the test procedure document. Positive, Negative and weak Positive Controls were determined in duplicates.

Assay principle

The PrioCHECK® Toxoplasma Ab porcine is an indirect ELISA for the detection of antibodies against Toxoplasma gondii. The test follows a short four step ELISA protocol. Test samples are incubated in plates coated with Toxoplasma antigen at room temperature. Plates are then washed and an enzyme labeled anti-pig antibody is added. The signal is measured and if color develops the sample is positive for anti-Toxoplasma antibodies.

Reagent and sample dilution were performed as described in the test procedure document. The assay program for the **Crocodile** is listed on the last page.
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Results:

Calculation of results:

\[ \text{OD Sample} \times 100 / \text{OD Positive Control} = X \% \text{ positivity} \]

Validation criteria:

- The mean OD\textsubscript{450} of the Positive Controls must be > 1.2
- The mean percentage of positivity of the weak Positive Controls must be > 35%
- The mean OD\textsubscript{450} of the negative results must be < 0.15

<table>
<thead>
<tr>
<th>sample ID</th>
<th>OD</th>
<th>% positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>2.018</td>
<td>99</td>
</tr>
<tr>
<td>PC</td>
<td>2.055</td>
<td>101</td>
</tr>
<tr>
<td>wPC</td>
<td>0.993</td>
<td>49</td>
</tr>
<tr>
<td>wPC</td>
<td>0.918</td>
<td>45</td>
</tr>
<tr>
<td>NC</td>
<td>0.026</td>
<td>1</td>
</tr>
<tr>
<td>NC</td>
<td>0.031</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 2. Positive (PC), Negative (NC) and weak Positive (wPC) Controls were determined in duplicates. OD is OD\textsubscript{450-620}. The diagram shows positive (PC), Negative (NC) and weak Positive (wPC) controls in relation to the measured OD\textsubscript{450-620} values.

Figure 3. The Diagram shows the Relation between the % positivity of confirmed positive and negative Samples.
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Summary:

All test criteria have been fulfilled. The mean OD_{405nm} of the Positive Controls determined in this test is 2.04. The mean percentage of positivity of the weak Positive Controls is 47% with a mean OD_{405nm} of the Negative results of 0.03. All 20 confirmed positive samples were identified correctly, showing the diagnostic sensitivity by using the combination Crocodile miniWorkstation and ELISA test kit PrioCHECK® Toxoplasma Ab porcine.

All 70 confirmed negative samples were identified correctly, showing the diagnostic specificity by using the combination Crocodile miniWorkstation and ELISA test kit PrioCHECK® Toxoplasma Ab porcine.

Conclusions:

Using the Crocodile for the assay procedure is extremely simple and involves only the addition of the samples. The Crocodile miniWorkstation is excellently suitable for use with the ELISA test kit PrioCHECK® Toxoplasma Ab porcine. This Application note demonstrates, that diagnostic sensitivity and specificity of the kit was fully achieved using the Crocodile miniWorkstation.

Acknowledgement:

We wish to thank Prionics AG for the supply of reagents and Pascal Schacher, Mario Purro and Daniel Zwald for their technical support.

www.prionics.com

www.titerkek-berthold.com
## Assay Program

<table>
<thead>
<tr>
<th>#</th>
<th>Step Name</th>
<th>Description</th>
</tr>
</thead>
</table>
| 1  | Incubate1       | Incubation Incubator On
Temperature: 22.3 °C Duration: 01:00:00                                    |
| 2  | Prime Wash1     | Washing Method: Prime Dispenser
Wash Solution Inlet: 1
| 3  | Wash1           | Washing Method: Soak Wash
Wash Solution Inlet: 1 Wash Fluid
Cycles: 4 Volume: 300ul Dispenser Depth: 1500 Aspiration Depth: 3000
Sweep: 5mm @ 1mm/s Count: 96 |
| 4  | Conjugate 2 Dispensing | Volume 800ul Inlet 2 Label "Conjugate可怕 Message" Method: Priming Count: 1 |
| 5  | Conjugate 2 Dispensing | Volume 100ul Inlet 2 Label "Conjugate可怕 Message" Method: Standard Count: 96 |
| 6  | Incubate2       | Incubation Incubator On
Temperature: 22.3 °C Duration: 01:00:00                                    |
| 7  | Wash2           | Washing Method: Soak Wash
Wash Solution Inlet: 1 Wash Fluid
Cycles: 4 Volume: 300ul Dispenser Depth: 1500 Aspiration Depth: 3000
Sweep: 5mm @ 1mm/s Count: 96 |
| 8  | Manual1         | check for remaining liquid
Duration: 00:02:00 Mode: Auto Continue Position: Insert Position |
| 9  | Prime TMB 3     | Dispensing Volume 800ul Inlet 3 Label "TMB可怕 Message" Method: Priming Count: 1 |
| 10 | TMB 3           | Dispensing Volume 100ul Inlet 3 Label "TMB可怕 Message" Method: Standard Count: 96 |
| 11 | Incubate3       | Incubation Incubator On
Temperature: 22.3 °C Duration: 00:15:00                                    |
| 12 | Prime Stop 4    | Dispensing Volume 800ul Inlet 4 Label "Stop可怕 Message" Method: Priming Count: 1 |
| 13 | Stop 4          | Dispensing Volume 100ul Inlet 4 Label "Stop可怕 Message" Method: Standard Count: 96 |
| 14 | Shake1          | Shaking for 00:01:00 at Shaker Position with 1mm Amplitude at 20Hz           |
| 15 | Measure1        | Reading Reference Measurement
Filter 1: 450nm (Pos:2) Filter 2: 620nm (Pos:4) Count: 96 |