

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 Sarcocystiidae. *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

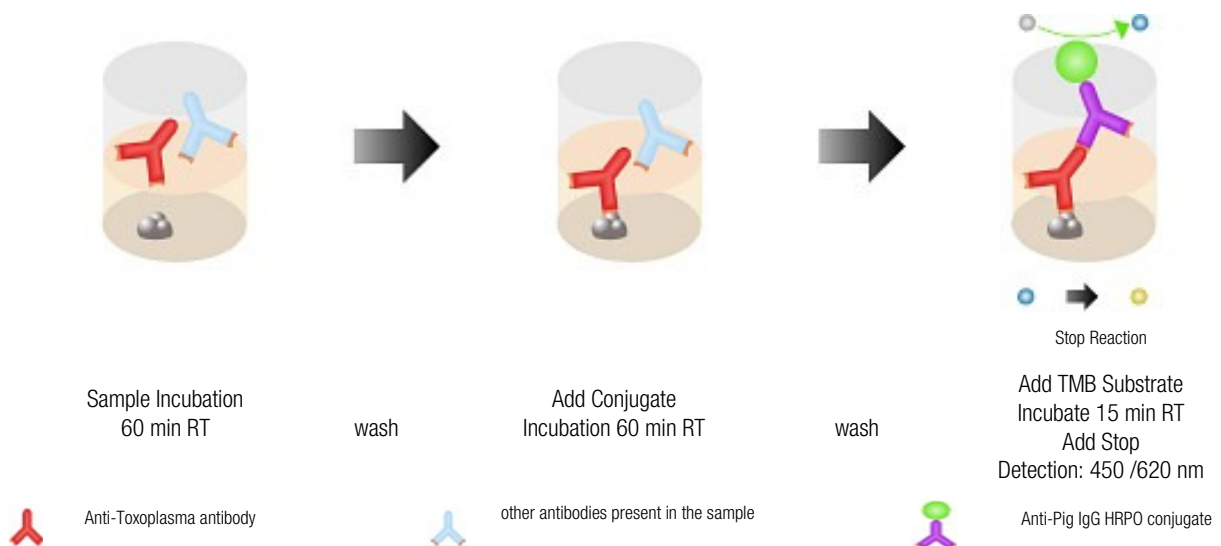


Figure 1. Schematic diagram of the procedural steps of the ELISA reaction. The ELISA kit from Prionics and was performed as described in the kit instructions. The absorbance of each well was measured at 450 nm with a reference measurement at 620 nm.

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:

$OD \text{ Sample} * 100 / OD \text{ Positive Control} = X \% \text{ positivity}$

Validation criteria:

The mean OD_{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_{450} of the negative results must be $< 0,15$

sample ID	OD	% positivity
PC	2,018	99
PC	2,055	101
wPC	0,993	49
wPC	0,918	45
NC	0,026	1
NC	0,031	2

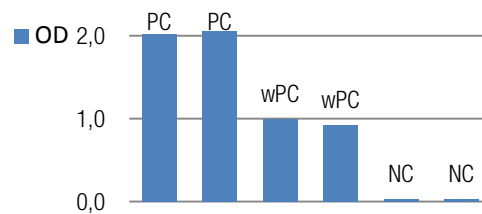


Figure 2. Positive (PC), Negative (NC) and weak Positive (wPC) Controls were determined in duplicates. OD is $OD_{450-620}$. The diagram shows positive (PC), Negative (NC) and weak Positive (wPC) controls in relation to the measured $OD_{450-620}$ values.

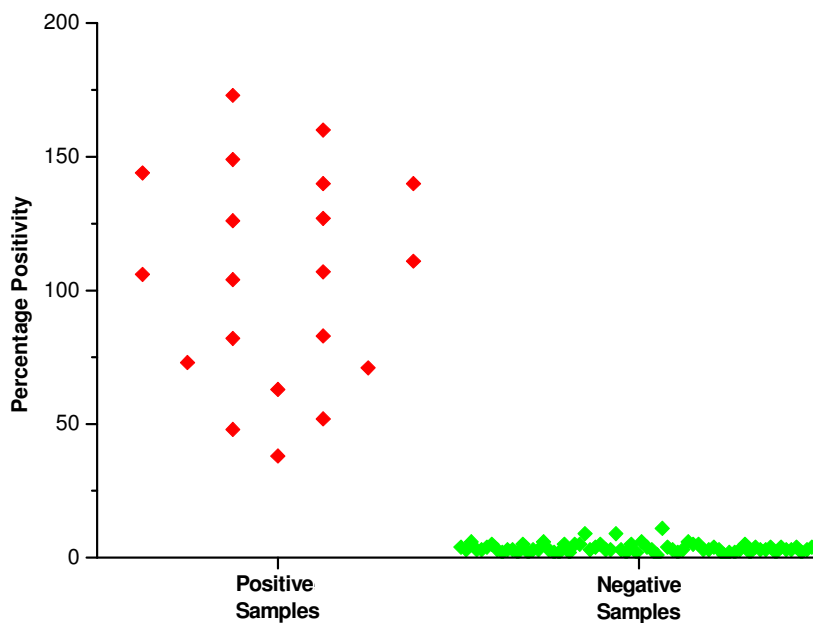


Figure 3. The Diagram shows the Relation between the % positivity of confirmed positive and negative Samples .

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Summary:

All test criteria have been fulfilled. The mean OD₄₅₀₋₆₂₀ 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 OD₄₅₀₋₆₂₀ 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 Pürro and Daniel Zwald for their technical support.



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Assay Program

#	Step Name	Description
1	Incubate1	Incubation Incubator On Temperature: 22.3 °C Duration: 01:00:00
2	Prime Wash1	Washing Method: Prime Dispenser Wash Solution Inlet: 1 Cycles: 7 Volume: 1000ul Dispenser Depth: 1300 Aspiration Depth: 1300 Count: 96
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	Prime Conjugate 2	Dispensing Volume 800ul Inlet 2 Label "Conjugate " Method: Priming Count: 1
5	Conjugate 2	Dispensing Volume 100ul Inlet 2 Label "Conjugate " 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 " Method: Priming Count: 1
10	TMB 3	Dispensing Volume 100ul Inlet 3 Label "TMB " 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 " Method: Priming Count: 1
13	Stop 4	Dispensing Volume 100ul Inlet 4 Label "Stop " 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