Berthold Detection Systems GmbH
Bleichstrasse 56–68
D-75173 Pforzheim/Germany

Phone: +49(0)7231/9206-0
Fax: +49(0)7231/9206-50
E-Mail: contact@titertek-berthold.com
Internet: www.titertek-berthold.com

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

Diagnostic Sensitivity and Specificity using Crocodile miniWorkstation

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

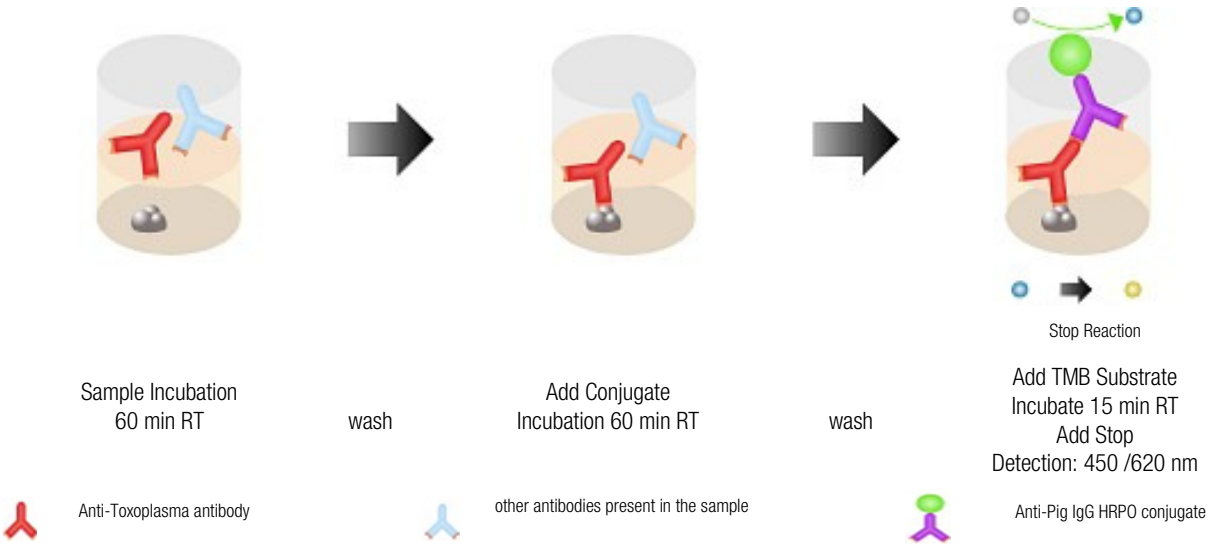

Figure 1. Schematic diagram of the procedural steps of the ELISA reaction. The ELISA kit from Prionics and was performed as described in the kit instructions. The absorbance of each well was measured at 450 nm with a reference measurement at 620 nm.

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.

Diagnostic Sensitivity and Specificity using Crocodile miniWorkstation

Results:

Calculation of results:

OD Sample*100 / OD Positive Control = X % 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

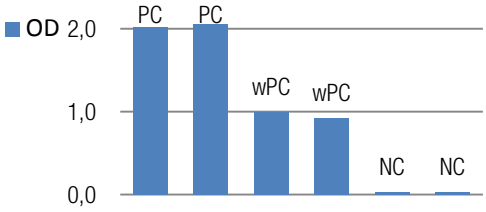

Figure 2. Positive (PC), Negative (NC) and weak Positive (wPC) Controls were determined in duplicates. OD is $OD_{450-620}$. The diagram shows positive (PC), Negative (NC) and weak Positive (wPC) controls in relation to the measured $OD_{450-620}$ values.

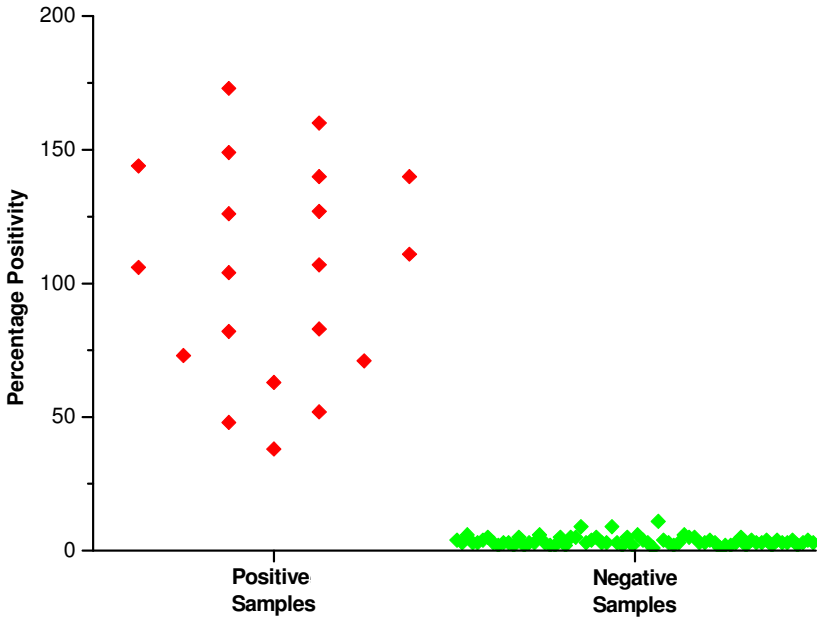

Figure 3. The Diagram shows the Relation between the % positivity of confirmed positive and negative Samples .

Diagnostic Sensitivity and Specificity using Crocodile miniWorkstation

Summary:

All test criteria have been fulfilled. The mean $OD_{450-620}$ 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 $OD_{450-620}$ 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.

HEALTHY ANIMALS, SAFE FOOD.

www.prionics.com

www.titertek-berthold.com

TITERTEK BERTHOLD

Diagnostic Sensitivity and Specificity using Crocodile miniWorkstation

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