APPLICATION NOTE

ANALYTICAL SENSITIVITY OF SAMPLES TESTED WITH THE CROCODILE MINIWORKSTATION IN COMPARISON TO HAND PROCESSING USING 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 worldwide, however, the prevalence in human and animal populations varies greatly among countries.

Materials:

Instrumentation:	Crocodile miniWorkstation Single channel pipette (20-200 µl)
Instrumentation for the manual test:	Tecan HydroFlexTM Tecan SunriseTM
Reagents:	PrioCHECK® Toxoplasma Ab porcine. Product N.: 7610230; Lot TX100401M; exp Date April 30th 2011 Demineralized water
Consumables:	Solution reservoirs Pipette tips

Method:

Test procedure

Analytical sensitivity is addressed by diluting positive samples and evaluating the dilution at which the samples can still be detected as positive. To determine the analytical sensitivity, two positive samples were diluted using serial dilutions from undiluted to 1:64. Both serial dilutions were run in triplicates using the Crocodile miniWorkstation, in parallel, duplicates were tested manually; a Tecan reader was used to measure the OD values from the manually processed samples.

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 labelled anti-pig antibody is added. The signal is measured and if colour 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.

Results:

Validation criteria:

The mean OD450 of the Positive Controls must be >1,2
The mean percentage of positivity of the weak Positive Controls must be > 35%
The mean OD450 of the Negative Controls must be < 0,15

sample ID	Crocodile	manual
PC	2,32	2,16
PC	2,3	1,99
wPC	1,01	0,92
wPC	1,01	0,91
NC	0,09	0,08
NC	0,1	0,08

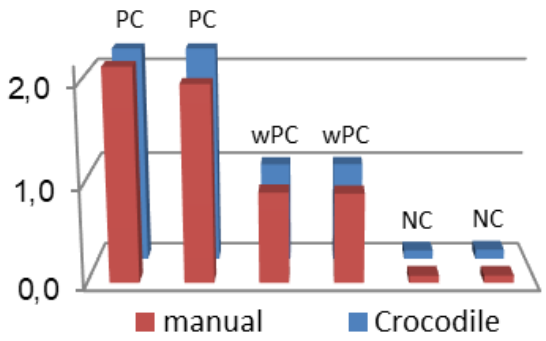

Figure 2: Positive (PC), Negative (NC) and weak Positive (wPC) Controls were determined in duplicates. OD is $OD_{450-620}$. The picture shows Positive (PC), Negative (NC) and weak positive (wPC) Controls in relation to the measured $OD_{450-620}$ values.

$OD_{450-620}$	Crocodile		manual	
Dilution Factor	**Vial 1**	**Vial 2**	**Vial 1**	**Vial 2**
1:1	2,11	2,08	2,06	1,97
1:2	1,30	1,25	1,23	1,17
1:4	0,68	0,65	0,66	0,63
1:8	0,39	0,38	0,38	0,36
1:16	0,22	0,22	0,22	0,21
1:32	0,15	0,15	0,14	0,14
1:64	0,12	0,10	0,11	0,10
NC	0,09	0,10	0,08	0,07

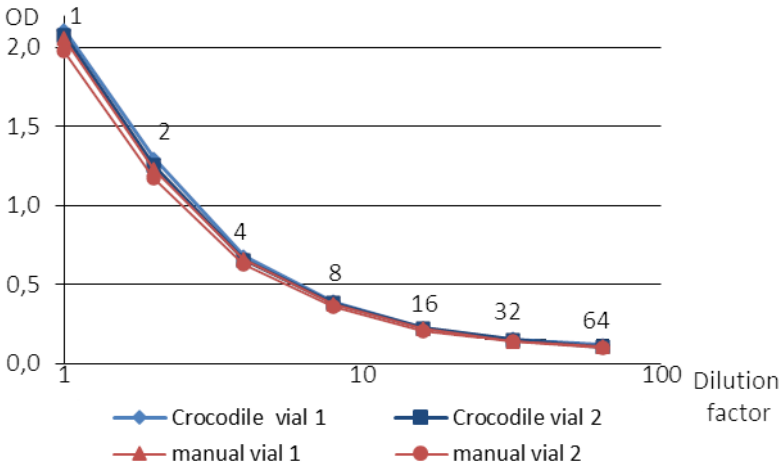

Figure 3: The table and the graph show the average results of $OD_{450-620}$ values of two different dilution series measured in triplicates (Crocodile) and duplicates (manual).

Summary:

The mean $OD_{450-620}$ values of samples analyzed with the **Crocodile** are comparable to the $OD_{450-620}$ values of samples processed manually.
Using the PrioCHECK® Toxoplasma Ab porcine a mean OD_{450} of < 0,15 is defined as negative. The Crocodile miniWorkstation was able to detect a dilution of 1:32 with a mean $OD_{450-620}$ value of 0,15, whereas for the manually processed samples the mean $OD_{450-620}$ value in this dilution was 0,14.

Conclusions:

Using the Crocodile miniWorkstation for the assay procedure is extremely simple and involves only the addition of the samples.
This application note demonstrates that the analytical sensitivity of the PrioCHECK® Toxoplasma Ab porcine using the Crocodile miniWorkstation is equivalent to analytical sensitivity achieved by manual processing of the test.

Acknowledgement:

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Assay Program:

#	Step Name	Description
1	**Incubate 1**	**Incubation** Incubator On Temperature: 22.3 °C Duration: 01:00:00
2	**Prime Wash 1**	**Washing** Method: Prime Dispenser Wash Solution Inlet: 1 Cycles: 7 Volume: 1000ul Dispenser Depth: 1300 Aspiration Depth: 1300 Count: 96
3	**Wash 1**	**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	**Incubate 2**	**Incubation** Incubator On Temperature: 22.3 °C Duration: 01:00:00
7	**Wash 2**	**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	**Manual 1**	**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	**Incubate 3**	**Incubation** Incubator On Temperature: 22.3 °C Duration: 00:15:00

#	Step Name	Description
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	**Shake 1**	**Shaking** for 00:01:00 at Shaker Position with 1mm Amplitude at 20Hz
15	**Measure 1**	**Reading** Reference Measurement Filter 1: 450 nm (Pos:2) Filter 2: 620 nm (Pos:4) Count: 96