Repeatability and Reproducibility using Crocodile miniWorkstation

Repeatability and Reproducibility of tests run on the **Crocodile** miniWorkstation 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 world wide, however, the prevalence in human and animal populations varies greatly among countries.

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

Repeatability is defined as the variation in measurements performed under the same conditions. For this purpose, one sample was tested in a 96-fold determination in the linear range of the assay. Repeatability is expressed as the coefficient of variation (CV) over the plate.

Reproducibility is defined as the ability to independently reproduce test results e.g. by testing in different plates on different days. For this purpose, 20 positive and 70 negative samples were tested in two independent runs.

Assay principle

Sample Incubation 60 min RT	wash	Add Conjugate Incubation 60 min RT	wash	Stop Reaction
				Add TMB Substrate Incubate 15 min RT Add Stop Detection: 450 / 620 nm

Anti-Toxoplasma antibody   other antibodies present in the sample   Anti-Pig IgG HRPO conjugate
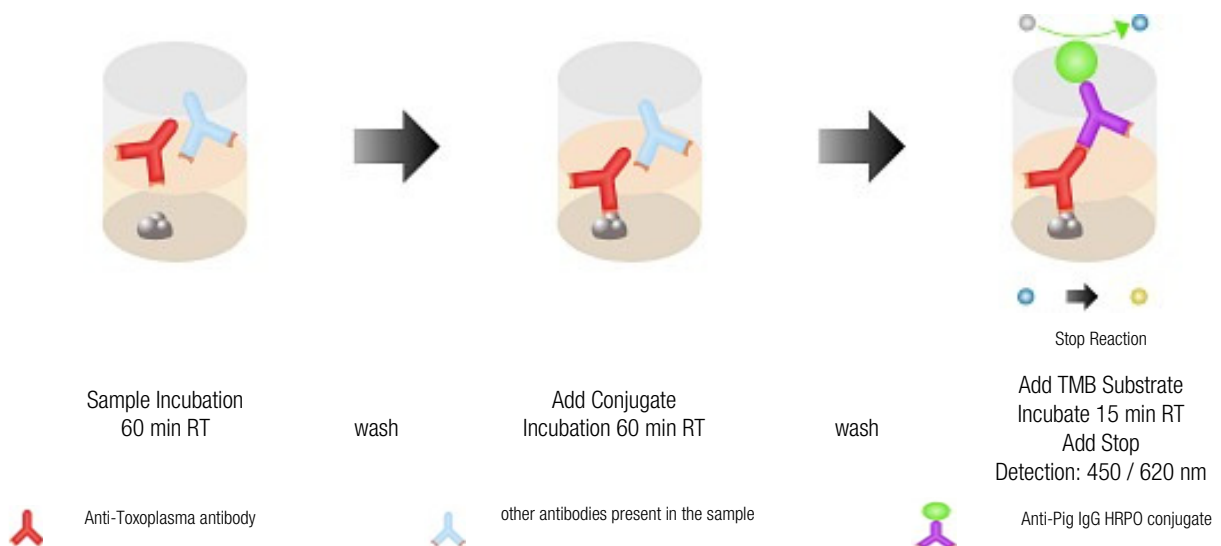
Figure 1. Schematic diagram of the procedural steps of the ELISA reaction. The ELISA kit from Prionics and was performed as described in the kit instructions. The absorbance of each well was measured at 450 nm with a reference measurement at 620 nm.

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 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:

Validation criteria for repeatability:

The standard deviation (SD) is the way of describing how dispersed a set of values are from the mean. The coefficient of variation (CV) is defined as the ratio of standard deviation to the mean. The CV is a standardization of the SD that allows comparison of variability of an assay.

For the PrioCHECK$^{®}$ Toxoplasma Ab porcine ELISA, the CV% of $OD_{450/620}$ values in one plate should be $< 8\%$.

OD	1	2	3	4	5	6	7	8	9	10	11	12
A	1,09	1,06	1,02	0,97	0,94	0,94	0,92	0,91	0,89	0,88	0,90	0,87
B	1,05	0,99	0,98	0,95	0,94	0,92	0,92	0,94	0,93	0,88	0,91	0,88
C	0,96	0,93	0,89	0,87	0,90	0,87	0,86	0,87	0,84	0,84	0,87	0,82
D	0,94	0,98	0,94	0,87	0,87	0,85	0,86	0,88	0,84	0,82	0,83	0,84
E	0,94	0,92	0,90	0,88	0,88	0,87	0,87	1,14	0,86	0,84	0,84	0,80
F	0,93	0,89	0,88	0,85	0,88	0,87	0,88	0,89	0,88	0,86	0,88	0,89
G	1,02	0,91	0,91	0,98	0,97	0,88	0,92	0,94	1,02	0,98	0,94	0,98
H	0,94	0,93	0,96	0,91	1,00	0,94	0,90	0,95	0,90	0,86	0,88	0,90

mean	SD	CV %
0,91	0,06	6,6

Figure 2. The first table shows $OD_{450/620}$ values of 96 samples. In the second table the corresponding mean $OD_{450/620}$ value, standard deviation and CV % are listed.

Validation criteria for reproducibility:

The linear correlation coefficient "r" measures the strength and the direction of a linear relationship between two measurements. A correlation between two independent tests greater than 0,8 is generally described as strong, whereas a correlation less than 0,5 is generally described as weak.

The coefficient of determination "r²" denotes the strength of the linear association between two tests. This coefficient is a measure of how well a regression line represents the percentage of the data that is closest to the line of the best fit.

A perfect correlation between two measurement would be indicated with an r = 1 and r² = 1.

For the PrioCHECK$^{®}$ Toxoplasma Ab porcine, a correlation "r" between two independent measurements must be > 0,8 with a coefficient of determination "r²" > 0,98.

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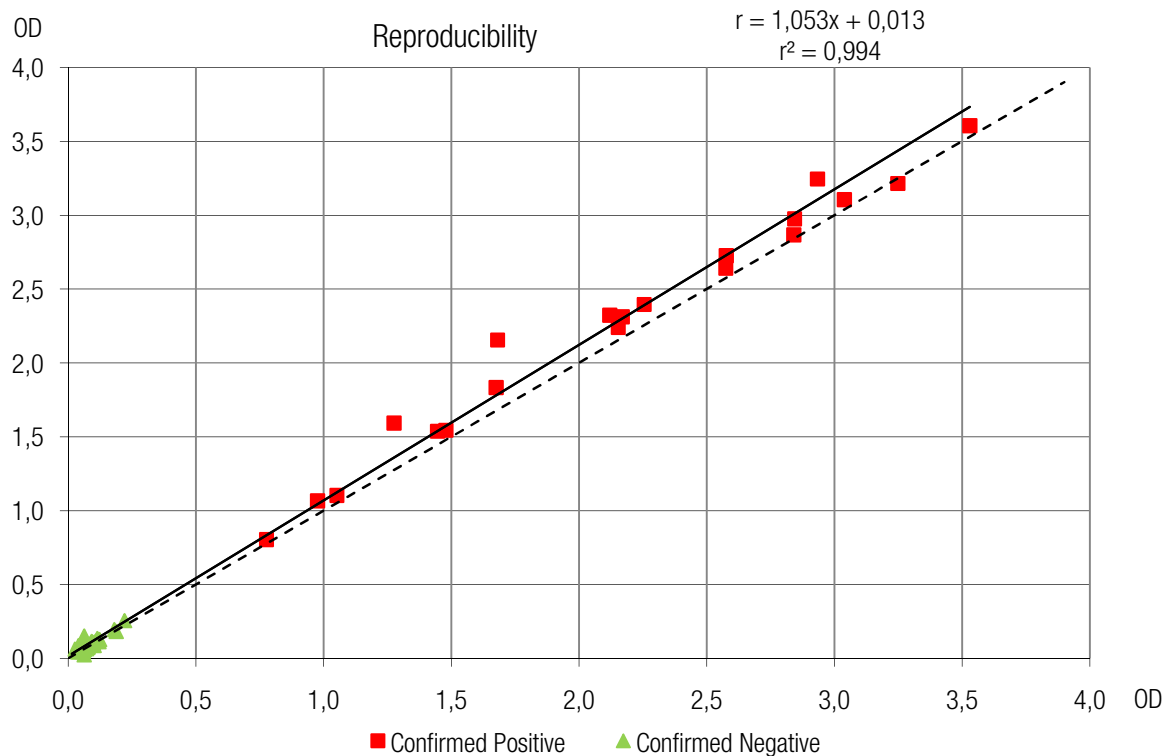# Repeatability and Reproducibility using Crocodile miniWorkstation

Figure 3. The graph shows the relation between the $OD_{450/620}$ values of two independent test runs on 20 confirmed positive and 70 confirmed negative samples. The linear correlation coefficient "r" (r= 1,053 + 0,013) measures the strength and the direction of a linear relationship between both measurements. The coefficient of determination "r²" (r² = 0,994) denotes the strength of the linear association between both tests.

Summary:

To test the repeatability of an assay processed by the **Crocodile** miniWorkstation, one positive sample was tested in 96-fold in one plate in the linear range of the assay with the PrioCHECK® Toxoplasma Ab porcine from Prionics. The resulting mean $OD_{450/620}$ value was 0,91 with a standard deviation of SD 0,06 and a calculated CV of 6,6% across the plate.

To test the reproducibility of an assay processed by the **Crocodile** miniWorkstation, 20 confirmed positive and 70 confirmed negative samples were tested in two independent runs together with duplicates of the Positive, weak Positive and Negative Controls with the PrioCHECK® Toxoplasma Ab porcine from Prionics. The linear correlation coefficient "r" was determined as r= 1,053 + 0,013 and the coefficient of determination "r²" as r² = 0,994.

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Conclusions:

This application note demonstrates the high repeatability and reproducibility using the **Crocodile** miniWorkstation in combination with the ELISA test kit PrioCHECK[®] Toxoplasma Ab porcine.

The repeatability, measured with a 96-fold determination in one plate, showed a very good CV (6,6).

The Crocodile miniWorkstation achieved a very good reproducibility of the $OD_{450/620}$ measurements. This is demonstrated by the resulting linear correlation coefficient of "r" with r = 1,053 + 0,013 and the coefficient of determination "r²" with r² = 0,994. A correlation between two independent tests greater than r = 0,8 is generally described as strong.

Acknowledgement:

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Assay Program

#	Step Name	Description
1	Incubate1	**Incubation** Incubator On Temperature: 22.3 ℃ 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 ℃ 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 ℃ 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