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ADAPTATION OF CIVTEST® CANIS LEISHMANIA FOR AUTOMATED ELISA

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INTRODUCTION

Canine leishmaniosis is a parasitic zoonotic disease caused by a flagellated protozoan of the genus *Leishmania*, transmitted by the bite of a female mosquito belonging to the subfamily *Phlebotominae* (genera *Phlebotomus* and *Lutzomyia*). Indirect immunofluorescence (IFAT) is the reference technique for the diagnosis of the disease. IFAT is a complicated technique that requires a certain degree of expertise for its interpretation. ELISA is an alternative technique which offers a suitable diagnostic performance and easy use and interpretation. CIVTEST® CANIS LEISHMANIA is an indirect ELISA, which shows an excellent level of correlation to IFAT.

The objective of this work is to adapt CIVTEST® CANIS LEISHMANIA to a high throughput automated ELISA platform.

MATERIALS AND METHODS

In order to adapt the system to the automated platform, it was essential to extend the time of the sample incubation from 10 to 30 minutes. For this propose a panel of 221 canine sera were analyzed by using both sample incubation protocols (10 minutes and 30 minutes). The status of the samples was defined by using CIVTEST® CANIS LEISHMANIA following the classical manual protocol (10 minutes sample incubation time) according to the manufacturer's instructions. The Rz values of each sample were calculated for each protocol. The correlation between Rz values obtained with each incubation time were analysed using a linear regression model. This model was later used to define the quantitative interpretation of results for the elongated protocol (adapted to the high throughput analysis system).

RESULTS AND DISCUSSION

The qualitative analysis showed a good R^2 value (0.8868, $p < 0.001$) indicating a high degree of correlation between both protocols.

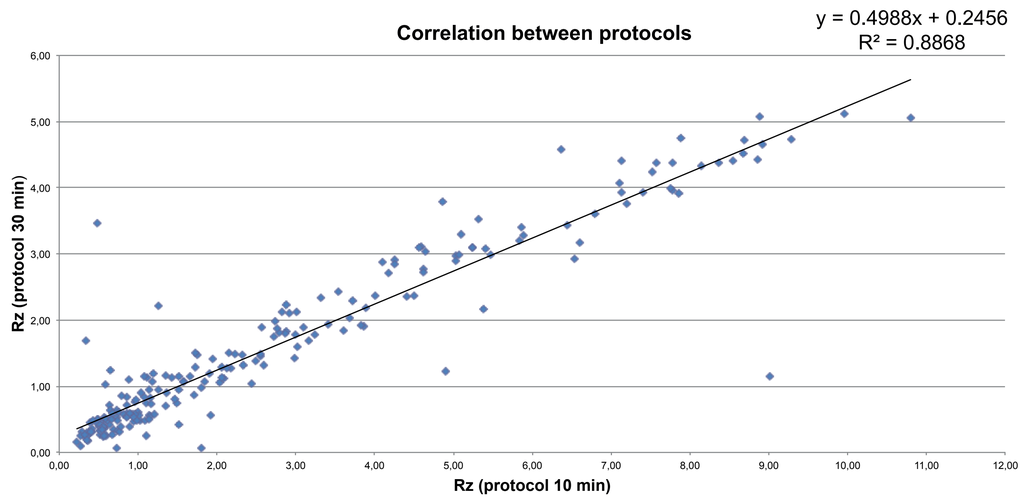

Figure 1. Correlation between both protocols and the linear regression model.

Using the linear regression model, new Rz interpretation values were calculated for the elongated protocol (30 minutes) from each Rz value of the short protocol (10 minutes). In this way, a new interpretation key for the kit was defined (see Table I). With this new interpretation key, the ELISA maintained the Se and Sp performance with regard to their correlation with IFAT titers previously defined for the short protocol.

Rz (protocol 10 min)	Rz (protocol 30 min)	Result	IFI Correspondence
Rz < 0.5	Rz < 0.5	Negative	Negative
0.5 <Rz < 0.7	0.5 <Rz < 0.6	Negative	1/20 to 1/40
0.7 < Rz < 0.9	0.6 < Rz < 0.7	Negative	1/40 to 1/80
0.9 < Rz < 1.1	0.7 < Rz < 0.8	Doubtful	1/80
1.1 < Rz < 1.5	0.8 < Rz < 1.0	Low Positive	1/80 to 1/160
1.5 < Rz < 2.0	1.0 < Rz < 1.2	Positive	1/160 to 1/320
2.0 < Rz < 3.0	1.2 < Rz < 1.8	High Positive	1/320 to 1/640
Rz > 3.0	Rz > 1.8	Very High Positive	> 1/640

Table 1. Interpretation values for the two protocols of CIVTEST® CANIS LEISHMANIA.

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