INgezim® PPA DAS 2.0

R.11.PPA.K.2

Double antibody ELISA for African Swine Fever Virus detection.
Whole blood, cell culture and spleen swine samples.

TECHNICAL INFORMATION

LAST REVISION: 10/07/23

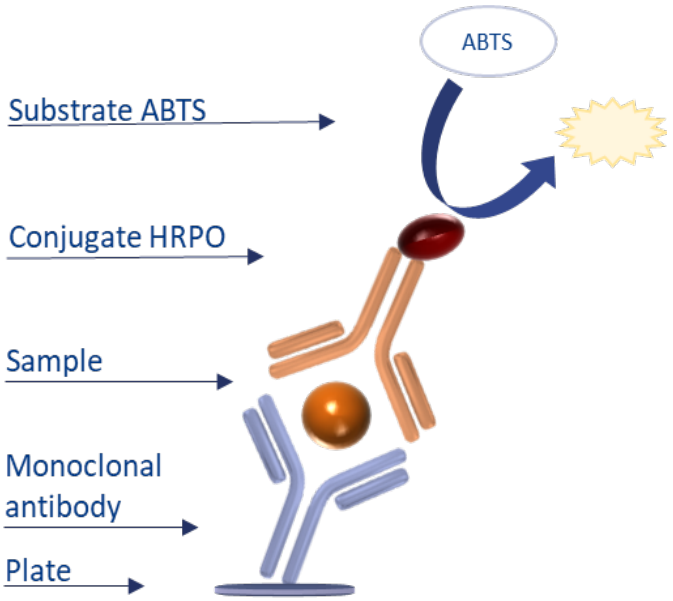
INDEX OF CONTENTS

1 PRODUCT APPLICATION

INgezim® PPA DAS 2.0 has been designed for the detection of African Swine Fever Virus (ASFV) antigen, in whole blood, cell culture or spleen samples.

2 TECHNICAL BASIS OF THE PRODUCT

The assay is based on double antibody ELISA technique, which scheme is briefly described hereunder:



1. Plates are supplied coated with ASFV specific Monoclonal Antibody (MAb) (protein vp72). Samples are added and incubated.
2. If samples contain ASFV antigen, it will bind the coated antibody.
3. At this point, a washing step is necessary to remove any non-specifically bound material.
4. When a monoclonal antibody specific to vp72 ASFV is added, it will bind the antigen if the sample is positive. The monoclonal antibody has been conjugated with HRPO for future detection.
5. Again, a washing step is necessary after incubation with the conjugate to remove any non-specifically bound material.
6. When a specific peroxidase substrate is added, if the sample is positive, colorimetric reaction will appear.

3 KEY REAGENTS USED

The optimal performance of the assay is mainly due to the quality of the key reagents, which are briefly described below:

- Monoclonal antibody specific to vp72 of ASFV, used as coating.
- Monoclonal antibody specific to vp72 of ASFV conjugated with peroxidase, used as conjugate.

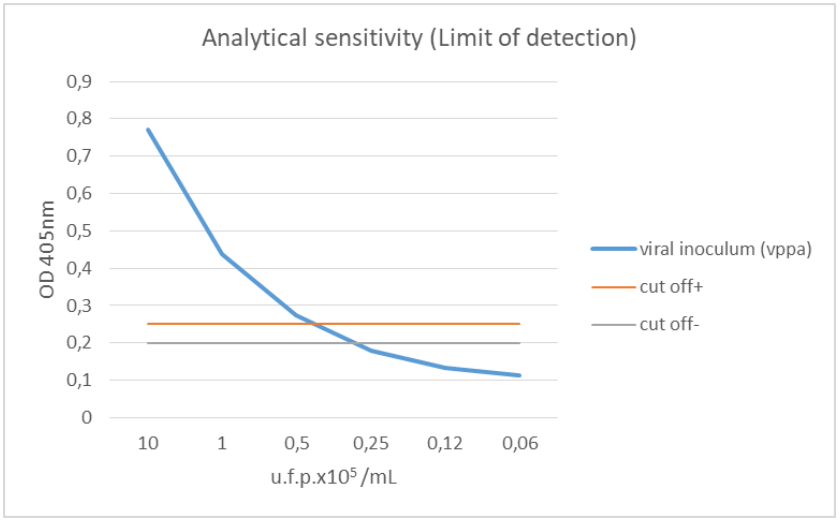
4 VALIDATION

4.1 ANALYTICAL SENSITIVITY

In order to determine the different aspects regarding analytical sensitivity, ASFV inoculum as well as samples from animals experimentally infected with different isolates were used.

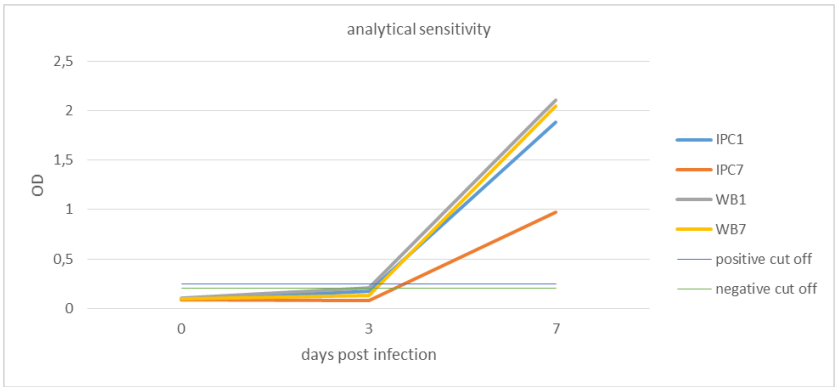
4.1.1 Detection limit

Different amounts of u.f.p./ml (plate forming units/ml) of ASFV inoculum were titrated. The obtained results indicated that the assay is capable of detecting $5x10^4$ u.f.p/mL of ASFV.



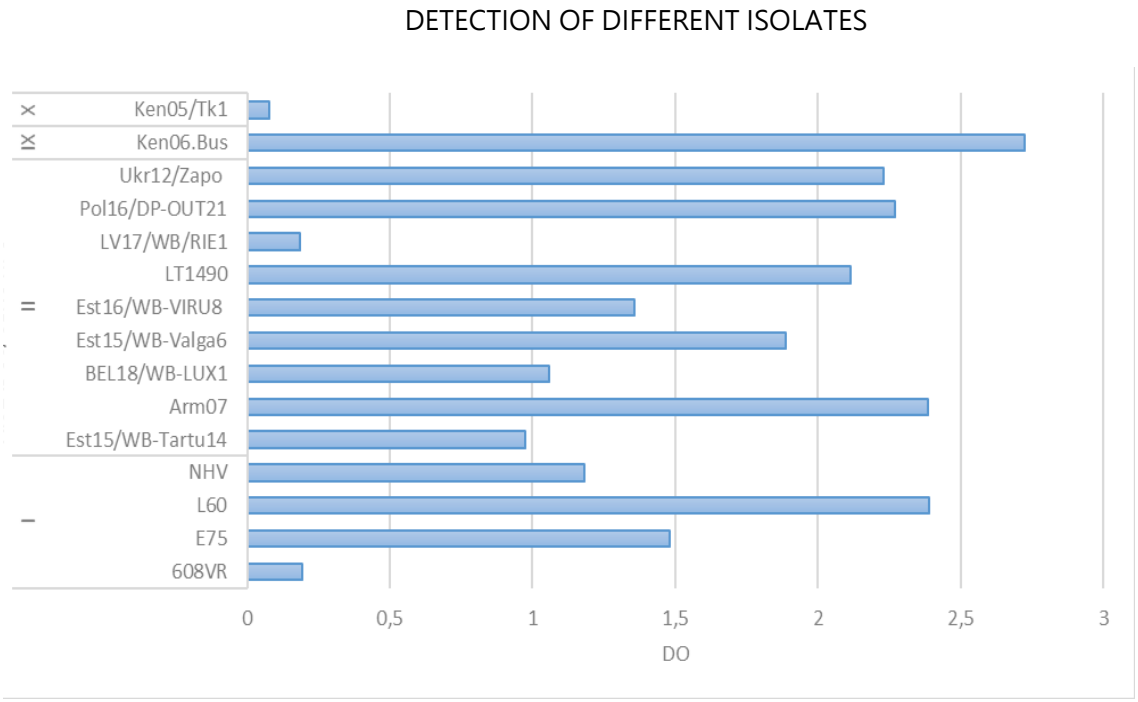
4.1.2 Precocity of the assay

Whole blood samples from 4 experimentally inoculated animals were analysed. Samples were extracted at different days post-infection (0, 3, 7). The obtained results indicated that the assay is capable of detecting ASFV antigen from day 7 post-infection.



4.1.3 Capacity of detection of different isolates

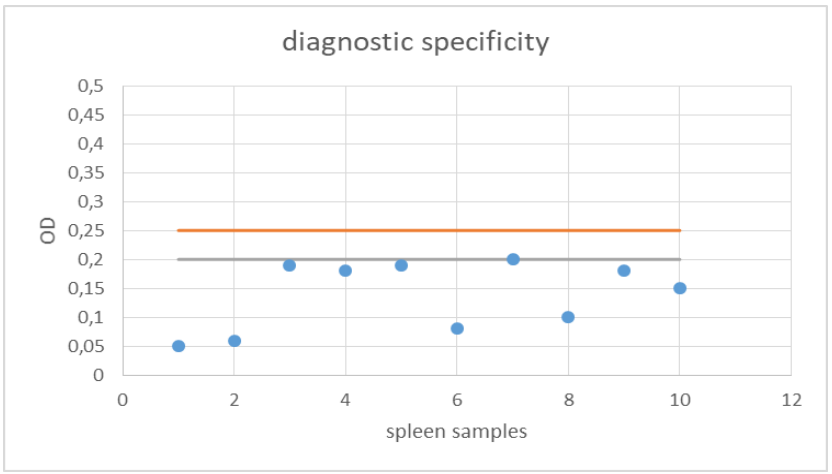
Whole blood samples from animals experimentally inoculated with different isolates belonging to different ASFV genotypes were analysed. The obtained results indicated that the assay is capable of detecting different ASFV isolates. The Ken05/Tk1 isolate belonging to genotype X corresponds to a sample collected at day 70 p.i. and showed Ct values of 36.93, which are very close to the cut-off point (0.4).



4.2 DIAGNOSTIC SPECIFICITY

4.2.1 Spleen samples

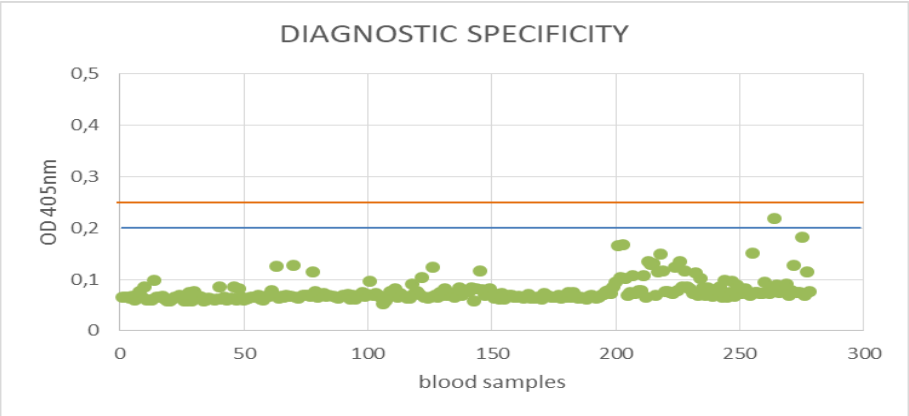
A panel of spleen samples from uninfected animals was analysed. The figure shows an example of the results obtained.



4.2.2 Whole blood samples

4.2.2.1 Internal study

In order to determine the diagnostic specificity of the assay with blood samples, 278 negative blood samples from Spain (ASFV free country) were analysed. All of them were also analysed by INgezim® ASFV crom Ag, confirming their negativity status. The obtained results indicated 99.6% specificity. The discordant sample was doubtful in the assay.



4.2.2.2 External study

This study was carried out by the CISA-INIA laboratory (Valdeolmos, Madrid).

1 collection of 33 blood samples negative by PCR-UPL and isolation, was analysed.

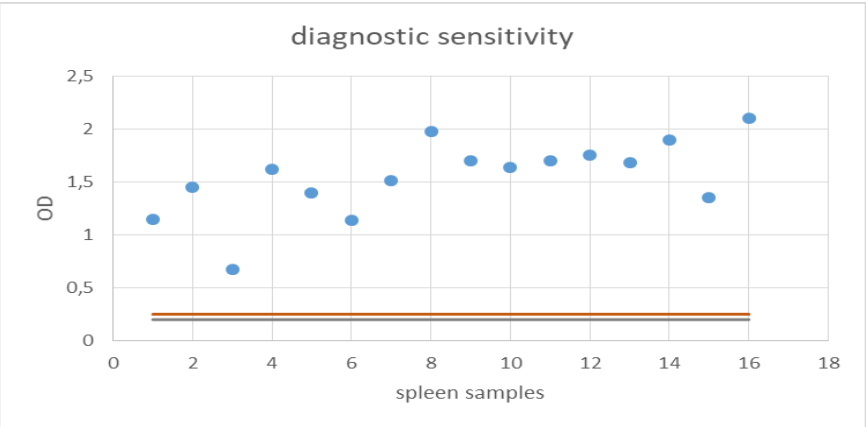
The obtained results indicated 97% specificity with respect to PCR-UPL and isolation in this study.

		INgezim® PPA DAS 2.0		
UPL-PCR		positive	doubtful	negative
	positive	0	0	0
	negative	0	3	30
	total	0	3	30

4.3 DIAGNOSTIC SENSITIVITY

4.3.1 Spleen samples

A panel of spleen samples from infected animals was analysed. The figure shows an example of the results obtained.



4.3.2 Whole blood samples (external study)

In order to determine the diagnostic sensitivity, the EU Reference Laboratory (CISA-INIA) carried out a study using 146 blood samples from animals experimentally infected with different isolates and positive by UPL-PCR:

- 67 from a kinetic study in which they were also analysed by isolation
- 79 coming from sample storage

The obtained results indicated 54.8% sensibility of the assay with respect to UPL-PCR.

In a more specific sensitivity study by Ct ranges or type of clinic, the obtained results are as shown below.

The premises of the study are:

- UPL-PCR<40 positive
- INgezim® PPA DAS 2.0 >0,25 positive

Ct Range	**UPL-PCR versus** INgezim® PPA DAS 2.0		*k index [95% CI].*
	Pos. UPL	*Pos. Ingezim® PPA DAS 2.0*	
< 20	51	50	98%
≥ 20 and < 25	32	20	62%
≥ 25 and < 30	25	6	24%
≥ 30 and < 35	20	3	15%
≥ 35 and < 40	18	1	5%
TOTAL	146	80	54,8%

Clinical form	**UPL-PCR versus** INgezim® PPA DAS 2.0		*k index [95% CI].*
	Pos. UPL	*Pos. Ingezim® PPA DAS 2.0*	
ACUTE	66	58	**87,8%**
SUBACUTE	26	9	**34%**
CHRONIC	51	12	**23,5%**
SUBCLINICAL	3	1	**33%**
TOTAL	146	80	**54,8%**