INgezim® Influenza A

R.10.FLU.K.3

Blocking ELISA kit for detection of antibodies specific to influenza type A virus nucleoprotein.
Multispecies assay for birds, porcine and equine serum samples.

TECHNICAL INFORMATION

LAST REVISION: 19/07/23

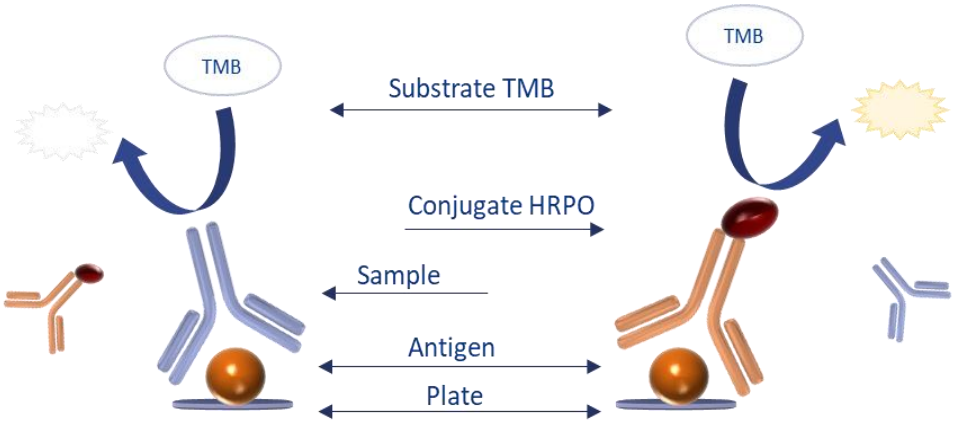
INDEX OF CONTENTS

Digitally signed by Bolea Petru
Date: 2025.07.10 16:24:58 EEST
Reason: MoldSign Signature
Location: Moldova
MOLDOVA EUROPEANĂ

1 PRODUCT APPLICATION

INgezim® Influenza A has been designed for the detection of specific antibodies to influenza type A virus nucleoprotein in poultry, swine and horse serum samples. With this technique, any type of immunoglobulins specific to influenza type A virus nucleoprotein can be detected.

2 TECHNICAL BASIS OF THE PRODUCT



1. Plates are supplied coated with Influenza type A virus antigen.
2. On these wells, samples are added and incubated.
3. If serum samples contain specific antibodies to nucleoprotein of Influenza A virus, they will bind the antigen coated to the wells.
4. At this point a washing step is needed to remove all non-specifically fixed materials.
5. When a Monoclonal Antibody specific of the nucleoprotein of Influenza A virus conjugated with HRPO is added, it will bind the antigen coated to the plate if there not antibodies specific in the sample (negative samples). In case there were antibodies specific in the sample (positive samples), these antibodies will block the specific sites avoiding the union of the conjugate.
6. Again, a washing step is required to remove all non-specifically fixed conjugate.
7. If a substrate for peroxidase is added, colorimetric reaction will appear for negative samples.

3 KEY REAGENTS USED

The optimum performance of the test is partially due to the high quality of the key reagents used in its formulation, which are:

- **Antigen**: Influenza A virus grown in cell cultures.
- **Conjugate:** Monoclonal Antibody specific to influenza virus type A nucleoprotein, conjugated with peroxidase.

4 VALIDATION

4.1 POULTRY

4.1.1 Sensitivity

In order to determine the sensitivity of the assay, sera from different types of vaccinated birds were analyzed:

- 45 chickens experimentally vaccinated with different vaccines (Vaccine 2: H7N4, Vaccine 3: H5N9, Vaccine 4: H5N9+H7N4, Vaccine 6: H7N2, Vaccine 7 H5N9+H7N4, Vaccine 8: H5N9+H7N1) and different number of doses.
- 2 turkeys experimentally vaccinated (Vaccine 1: H5N9+H7N4 and Vaccine 5: H5N9+H7N1).
- 20 vaccinated birds from the Zoo.

All the 67 samples were positive by INgezim® Influenza A. 7 chickens that received a single dose, all of them showed high positivity at 50 dpv (no previous samples available). Furthermore, the fact that all samples were positive indicates that the assay is useful for the detection of specific antibodies to different strains of influenza A.

Some of the results that were obtained are showed below.

The results that were obtained with these samples in this study indicated >99% sensitivity.

SAMPLE	REFERENCE	Abs 405 nm	% Competition	Result
Zoo 15860	1 (tratado)	0,226	85	POS
	2	0,242	83	POS
	3	0,154	89	POS
	4	0,145	90	POS
	5	0,082	94	POS
	6	0,26	82	POS
	7	0,156	89	POS
	8	0,132	91	POS
	9	0,098	93	POS
	10	0,239	84	POS
	11	0,712	51	POS
	12	0,431	71	POS
	13	0,237	84	POS
	14	0,577	61	POS
	15 (tratado)	0,104	93	POS
	16 (tratado)	0,432	71	POS
	17	0,267	82	POS
	18 (tratado)	0,074	95	POS
	19	0,371	75	POS
	20	0,32	78	POS
Control Neg	**C**	**1,436**	**0**	**NEG**
Control Pos	**DX+85**	**0,338**	**76**	**POS**

MUESTRA	REFERENCIA	Abs 405 nm	% Competición	Resultado
Vaccinated chicken doses 1st	Vaccine 2	0,152	89	POS
	Vaccine 3	0,11	92	POS
	Vaccine 4	0,072	95	POS
	Vaccine 6	0,067	95	POS
	Vaccine 7	0,079	94	POS
	Vaccine 8	0,074	95	POS
Vaccinated turkeys 1st doses	Vaccine 1	0,11	92	POS
	Vaccine 5	0,151	89	POS
Control Neg	F158	1,395	100	NEG
Control Pos	DX+85	0,105	93	POS
Vaccinated chickens with H5N9 + H7N1.	1 One doses	0,285	83	POS
	2 One doses	0,388	76	POS
	3 One doses	0,253	85	POS
	4 One doses	0,343	79	POS
	5 One doses	0,275	83	POS
	6 One doses	0,580	65	POS
	7 One doses	0,388	76	POS
	8 Two doses	0,069	96	POS
	9 Two doses	0,080	95	POS
	10 Two doses	0,100	94	POS
	11 Two doses	0,080	95	POS
	12 Two doses	0,080	95	POS
	13 Two doses	0,117	93	POS
	14 Two doses	0,097	94	POS
	15 Two doses	0,063	96	POS
	16 Two doses	0,071	96	POS
	17 Two doses	0,072	96	POS
	18 Two doses	0,066	96	POS
	19 Two doses	0,084	95	POS
	20 Two doses	0,096	94	POS
	21 Two doses	0,079	95	POS
	22 Two doses	0,064	96	POS
	23 Two doses	0,066	96	POS
	24 Two doses	0,092	94	POS
	25 Two doses	0,072	96	POS
	26 Two doses	0,070	96	POS
	27 Two doses	0,086	95	POS
	28 Two doses	0,082	95	POS
	29 Two doses	0,084	95	POS
	30 Two doses	0,144	91	POS
	31 Two doses	0,144	91	POS
	32 Two doses	0,144	91	POS
	33 Two doses	0,080	95	POS
	34 Two doses	0,087	95	POS
	35 Two doses	0,091	94	POS
	36 Two doses	0,130	92	POS
	37 Two doses	0,112	93	POS
Control Neg	C	1,642	-14,3	NEG
Control Pos	DX+85	0,306	79	POS

4.1.2 Specificity

In order to determine the specificity of the assay, sera from different origins were analyzed, all of them previously classified as negative using different techniques:

- 94 turkeys
- 50 partridges
- 13 chickens
- 9 ostriches
- 24 wild birds
- 139 wild birds: 83 vultures, 4 flamingos, 1 kite y 51 Egyptian vulture (Doñana 1999)
- 284 farm chickens

The results that were obtained in this study indicated 98.23% specificity of the assay.

4.2 SWINE

4.2.1 Diagnostic sensitivity and specificity

291 swine samples were analysed:

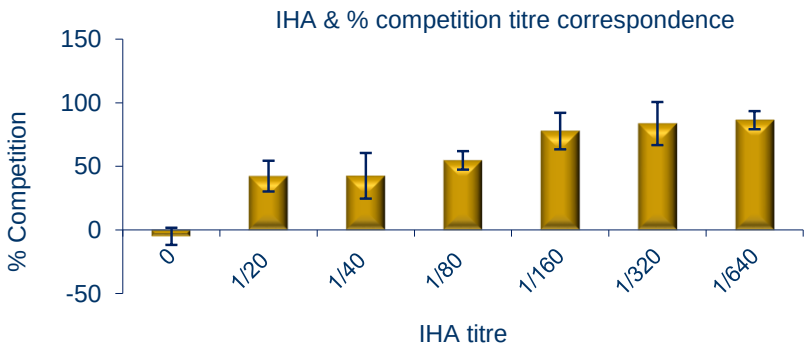
- 160 samples from animals experimentally vaccinated with H1N1, H1N2 and H3N2 strains and bled at days 28, 35, 42, 49 and 56.
- 41 control animals
- 90 farm animals

All of them were previously classified by the indirect ELISA test INgezim® Influenza Porcina. The results indicated that the assay could detect specific antibodies to the N protein of different strains of the INFLUENZA A virus, with a relative sensitivity and specificity compared to indirect ELISA of 94% and 98% respectively.

4.2.2 Correspondence with IHA

24 samples of animals inoculated with the H1N1, H1N2 or H3N2 strain or with the three strains mixed were analysed. These samples had previously been classified by IHA.

The results obtained indicate a good correlation between both techniques (92% correlation). It can also be observed that there is a correlation between the competition % and the IHA titre.



4.3 HORSE

4.3.1 Correspondence with IHA

142 horse samples with different IHA titres have been analyzed and compared with ID Screen® Influenza A. The results obtained are shown in the following table:

	IHA	
	Sen	Spe
INgezim®	97%	92%
ID Screen®	98%	73%