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USE OF THE ID-VET AVIAN INFLUENZA ELISA RANGE (NUCLEOPROTEIN, H5, H7, N1 OR N2) FOR THE DETECTION OF ANTIBODIES TO HAEMAGGLUTININ AND NEURAMINIDASE SUBTYPES IN AVIAN POPULATIONS

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Introduction and objectives. Influenza viruses are responsible for influenza diseases affecting humans and animals. The viral type A, B, or C is defined by the nature of the internal nucleoprotein (NP). The A type is the most conserved genus, affecting humans as well as avian, porcine and equine species. Subtype of avian influenza A viruses are characterised by their haemagglutination (H) and neuraminidase (N) surface proteins.

In recent years, outbreaks of avian influenza (AI) in Europe and around the world have led to an increased need for rapid, reliable diagnostic methods. The most serological monitoring programs for domestic and wild birds look first for the presence of antibodies against Avian Influenza A virus. In case of positive results, H5 and H7 subtypes must be excluded, as low pathogen H5Nx and H7Nx AIV subtypes may rapidly mute to high pathogenic forms. These confirmation tests are often carried out by haemagglutination inhibition test HI, a quite long and subjective method. The results strongly depend on the antigen used. N1 and N2 tests allow to detect possible presence of N1/N2 subtypes in vaccinated animals (DIVA strategy).

In this context, ID-VET has developed five different competitive ELISAs for the detection of specific antibodies:

- ✓ ID Screen® Influenza A ELISA (detects antibodies to the highly conserved Nucleoprotein - for all AI A virus subtypes)
- ✓ ID Screen® Influenza H5 ELISA (antibodies to Haemagglutinin H5)
- ✓ ID Screen® Influenza H7 ELISA (antibodies to Haemagglutinin H7)
- ✓ ID Screen® Influenza N1 ELISA (antibodies to Neuraminidase N1)
- ✓ ID Screen® Influenza N2 ELISA (antibodies to Neuraminidase N2)

This study evaluates the specificity and sensitivity of these tests.

Material and methods. The ID Screen® competitive ELISAs (NP, H5, H7, N1) were used according to the manufacturer's instructions. Briefly, specimens to be tested and controls are added to microwells coated with the specific protein. Anti-AI virus antibodies, if present, form an antibody-antigen complex which masks the specific epitopes. An anti-AIV-peroxidase conjugate is added to the microwells. It fixes to the remaining free epitopes, forming an antigen-conjugate-complex. After washing the substrate (TMB) is added. The resulting coloration depends on the quantity of specific antibodies present in the specimen to be tested. In the absence of antibodies, a blue coloration appears which becomes yellow after addition of the stop solution. In the presence of antibodies, no coloration appears. The optical densities (ODs) are read at 450 nm and results are expressed as S/N (sample / negative control ratio).

Specificity on negative populations. Samples from disease-free populations and vaccinated animals were tested on the different ELISA tests. For the detailed number of tested sera and the S/N% distributions see figures 1-6.

Neuraminidase and Haemaglutinin specificity and sensitivity. A large number of sera from vaccinated and/or infected animals of H5Nx, H7Nx, HxN1 and HxN2 subtypes were tested for sensitivity. Sera of other subtypes were tested for specificity. These sera were kindly provided by Fluaid consortium and different reference labs (Egypt, Italy and others).

Results. *Specificity.* The following specificities on disease-free animals or vaccinated animals were observed:

- ID Screen® Influenza A ELISA: 100% (CI95 99.36 - 100%)
- ID Screen® Influenza H5 ELISA: 100% (CI95: 94.22 - 100%).
- ID Screen® Influenza H7 ELISA:
  - 97,60% (IC95:96.62% – 98.31%) on vaccinated animals
  - 97,32% (IC95:91.79% – 99.3%) on disease-free animals

- ID Screen® Influenza N1 ELISA: 100% (IC95%: 99.24% - 100%)
- ID Screen® Influenza N2 ELISA: 97.26% (CI95: 94.15 – 98.74%)

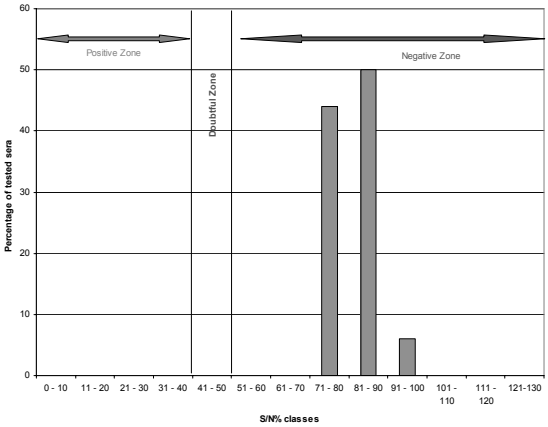

Fig. 1. ID Screen Influenza A Competition ELISA. S/N distribution for negative field sera (400 chicken, 100 turkey, 100 duck and ostrich. Total n=600 sera).

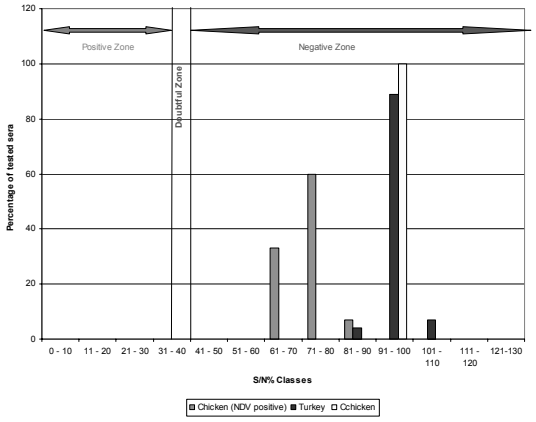

Fig. 2. ID Screen Influenza H5 Competition ELISA. S/N distribution for negative field sera (28 turkey, 36 chicken and 15 NDV-positive chicken. Total n=79 sera).

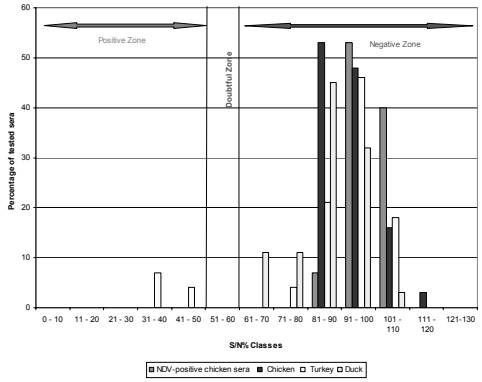

Fig. 3. ID Screen Influenza H7 Competition ELISA. S/N distribution for disease-free animals (28 turkey, 36 chicken, 38 ducks and 15 NDV positive chicken. Total n= 117 sera).

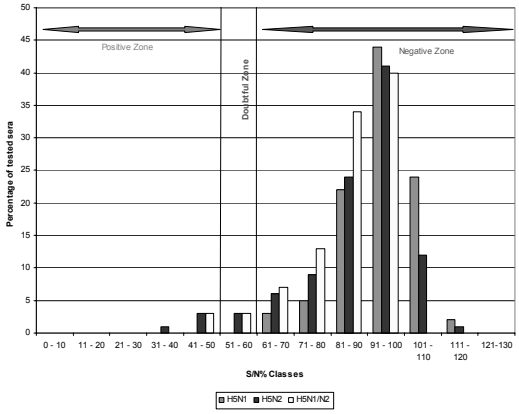

Fig. 4. ID Screen Influenza H7 Competition ELISA. S/N distribution for vaccinated animals (478 H5N2 vaccinated animals, 796 H5N1 vaccinated animals and 94 H5N1 / H5N2 vaccinated animals, total n=1368 bird sera).

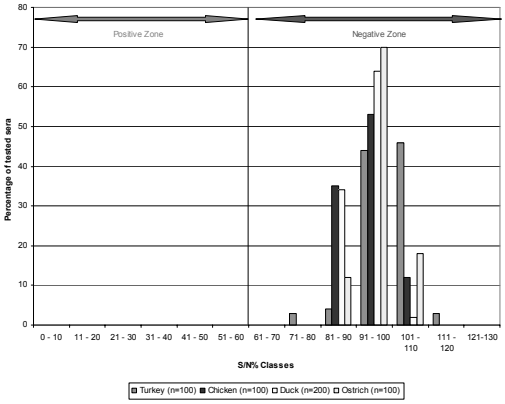

Fig. 5. ID Screen Influenza N1 Competition ELISA. S/N distribution for negative field sera tested (100 turkey, 100 chicken, 100 ostrich and 200 ducks. Total n=500 sera).

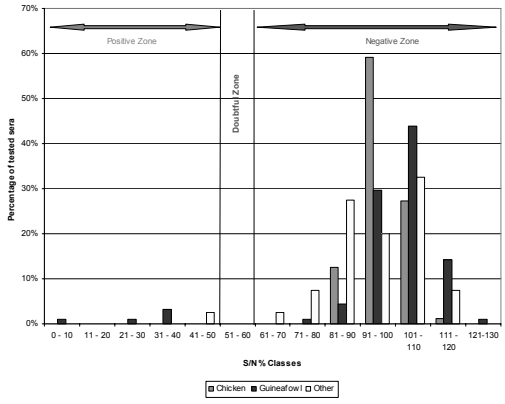

Fig. 6. ID Screen Influenza N2 Competition ELISA. S/N distribution for negative field sera tested (88 chicken, 91 guineafowls and 40 other birds infected by HxNy subtypes other than HxN2, total n= 219 sera).

Sensitivity. The ID Screen® NP, H5, N1 and N2 competitive ELISAs correctly identified all subtypes tested (except one borderline sample for H5). The H7 ELISA correctly identified all serotypes tested, although some weak cross-reactions were observed with H10Nx and H15Nx strains.

As an example results for ID Screen® Influenza H5 ELISA and Influenza N2 ELISA are illustrated in tables 1 and 2. For N1 results see reference.

Table 1 – Subtypes tested on ID Screen Influenza H5 ELISA. Cut-off 35/40%.

Sample	Subtype	S/P%	Status	Sample	Subtype	S/P%	Status
1	H7N7	53	NEG	37	H5N3	4	POS
2	H7N3	65	NEG	38	H5N3	6	POS
3	H7N7	54	NEG	39	H5N3	4	POS
4	H7N7	75	NEG	40	H5N3	3	POS
5	H7N7	71	NEG	41	H5N3	4	POS
6	H5N3	24	POS	42	H5N3	5	POS
7	H5N3	15	POS	43	H5N3	5	POS
8	H5N3	26	POS	44	H1N1	64	NEG
9	H5N3	18	POS	45	H1N1	39	DOUBT
10	H5N3	14	POS	46	H2N2	33	POS
11	H5N3	13	POS	47	H3N1	77	NEG
12	H5N3	14	POS	48	H3N8	58	NEG
13	H5N3	16	POS	49	H4N6	45	NEG
14	H5N3	15	POS	50	H6N2	57	NEG
15	H5N3	13	POS	51	H6N2	73	NEG
16	H5N1	2	POS	52	H7N7	63	NEG
17	H5NDV	4	POS	53	H8N4	78	NEG
18	H5NDV	3	POS	54	H9N2	67	NEG
19	H5N1	3	POS	55	H9N2	62	NEG
20	H5N9	23	POS	56	H10N7	73	NEG
21	H5N2	2	POS	57	H10N8	57	NEG
22	H5N1	6	POS	58	H11N6	52	NEG
23	H5N6	24	POS	59	H11N6	49	NEG
24	H5N3	7	POS	60	H12N5	75	NEG
25	H5N2	4	POS	61	H13N6	78	NEG
26	H5N3	5	POS	62	H14N5	60	NEG
27	H5N3	4	POS	63	H15N9	73	NEG
28	H5N3	10	POS	64	H16N3	61	NEG
29	H5N3	5	POS	65	H16N3	78	NEG
30	H5N3	8	POS	66	Neg 1	88	NEG
31	H5N3	9	POS	67	Neg 2	86	NEG
32	H5N3	6	POS	68	Neg 3	92	NEG
33	H5N3	4	POS	69	Neg 4	81	NEG
34	H5N3	8	POS	70	Neg 5	82	NEG
35	H5N3	3	POS	71	Neg 6	76	NEG
36	H5N3	5	POS	72	Neg 7	75	NEG

Field Sentinel infected with H5N2. NV; Non vaccinated. V; Vaccinated (H5N9/H7N1).

Discussion and conclusions. The ID VET Influenza ELISAs allow for the rapid screening of avian populations, both for Influenza A via the nucleoprotein ELISA, or for H5, H7, N1 or N2 specific antibodies.

Access to non-vaccinated, naturally-infected sera H5 and H7 sera is difficult due to the fact that infected animals are rapidly culled in the European Union. ID VET welcomes any collaborations with laboratories having such sera in their possession.

Acknowledgements. ID VET would like to thank the partners the Fluaid consortium for the validation of these different ELISA tests, particularly for N1 and N2.

Reference. Preliminary validation of a commercial Avian Influenza N1 antibody competitive ELISA that can be used as a part of a DIVA strategy. Dundon et al. Epizone meeting 2007.

Table 2 – Subtypes tested on ID Screen Influenza N2 ELISA. Cut-off 50/60%.

Sample	OD	S/N%	Status	Sample	OD	S/N%	Status
H1N1	1,186	89%	NEG	NV+ H5N2 (191)	0,161	12%	POS
H2N3	1,130	85%	NEG	NV+ H5N2 (192)	0,097	7%	POS
H3N8	1,176	89%	NEG	NV + H5N2 (193)	0,417	31%	POS
H4N8	1,129	85%	NEG	NV + H5N2 (198)	0,149	11%	POS
H5N1	0,997	75%	NEG	NV + H5N2 (199)	0,239	18%	POS
H5N2	0,054	4%	POS	NV +H5N2 (200)	0,338	25%	POS
H5N3	1,118	84%	NEG	Sent. 01 – H5N2	0,288	22%	POS
H5N9	0,830	63%	NEG	Sent. 02 – H5N2	0,228	17%	POS
H6N2	0,111	8%	POS	Sent. 03 – H5N2	0,237	18%	POS
H7N1	1,199	90%	NEG	Sent. 04 – H5N2	0,252	19%	POS
H7N3	1,069	81%	NEG	Sent. 05 – H5N2	0,249	19%	POS
H7N4	1,162	88%	NEG	Sent. 06 – H5N2	0,229	17%	POS
H8N4	1,243	94%	NEG	Sent. 07 – H5N2	0,593	45%	POS
H9N2	0,056	4%	POS	Sent. 08 – H5N2	0,148	11%	POS
H9N7	1,177	89%	NEG	Sent. 09 – H5N2	0,450	34%	POS
H10N1	1,289	97%	NEG	Sent. 10 – H5N2	0,125	9%	POS
H10N8	1,109	84%	NEG	Sent. 11 – H5N2	0,293	22%	POS
H11N6	1,251	94%	NEG	Sent. 12 – H5N2	0,233	18%	POS
H11N9	0,979	74%	NEG	Sent. 13 – H5N2	0,191	14%	POS
H12N5	1,175	89%	NEG	Sent. 14 – H5N2	0,310	23%	POS
H13N6	0,653	49%	DOUBT	Sent. 15 – H5N2	0,231	17%	POS
H14N5	1,044	79%	NEG	Sent. 16 – H5N2	0,228	17%	POS
H15N9	1,377	104%	NEG	Sent. 17 – H5N2	0,378	29%	POS
H16N3	1,395	105%	NEG	Sent. 18 – H5N2	0,472	36%	POS
SPF	1,340	101%	NEG	Sent. 19 – H5N2	0,299	23%	POS
NDV (Ulster)	1,373	104%	NEG	Sent. 20 – H5N2	0,268	20%	POS
NDV (Pigeon)	1,255	95%	NEG	Sent. 21 – H5N2	0,509	38%	POS
PMV2	1,254	95%	NEG	Sent. 22 – H5N2	0,300	23%	POS
PMV3 (Tk)	1,409	106%	NEG	Sent. 23 – H5N2	0,348	26%	POS
PMV3 (Parrot)	1,325	100%	NEG	Sent. 24 – H5N2	0,296	22%	POS
PMV4	1,433	108%	NEG	Sent. 25 – H5N2	0,461	35%	POS
PMV6	1,437	108%	NEG	Sent. 26 – H5N2	0,541	41%	POS
PMV7	1,232	93%	NEG	Sent. 27 – H5N2	0,342	26%	POS
PMV8	1,437	108%	NEG	Sent. 28 – H5N2	0,159	12%	POS
PMV9	1,356	102%	NEG	Sent. 29 – H5N2	0,253	19%	POS
EDS	1,323	100%	NEG	Sent. 30 – H5N2	0,210	16%	POS
M41	1,531	115%	NEG	Sent. 31 – H5N2	0,328	25%	POS
D-274	1,403	106%	NEG	Sent. 32 – H5N2	0,273	21%	POS
624-I	1,513	114%	NEG	Sent. 33 – H5N2	0,156	12%	POS
IT-02	1,577	119%	NEG	Sent. 34 – H5N2	0,125	9%	POS
793-B	1,411	106%	NEG	Sent. 35 – H5N2	0,232	17%	POS
QX	1,432	108%	NEG	Sent. 36 – H5N2	0,153	12%	POS
D-1466	1,377	104%	NEG				

ИСПОЛЬЗОВАНИЕ ELISA ТЕСТ-СИСТЕМ ПРОИЗВОДСТВА КОМПАНИИ ID-VET ДЛЯ ДИАГНОСТИКИ ГРИППА ПТИЦ С ЦЕЛЬЮ ОПРЕДЕЛЕНИЯ АНТИТЕЛ К РАЗЛИЧНЫМ ПОДТИПАМ ГЕМАГГЛЮТИНИНА И НЕЙРАМИНИДАЗЫ В ПОПУЛЯЦИЯХ ПТИЦ

Поркью Ф., Лессо С. Компания ID-VET, Монпелье, Франция.

В последние годы вспышки птичьего гриппа в Европе и во всем мире привели к возросшей необходимости разработки быстрых и достоверных диагностических методов. В связи с этим компания ID-VET разработала 5 различных наборов ИФА для определения специфических антител (нулеопротеин, H5, H7, N1 и N2). В статье представлены материалы по оценке специфичности и чувствительности этих наборов.