OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

Name of the diagnostic kit: BioChek Avian Influenza Antibody Test Kit
Manufacturer: BioChek UK Ltd
OIE Approval number: 20080203
Date of Registration: May 2008

Disease: Avian Influenza (AI)

Pathogen Agent: Type A avian influenza virus

Type of Assay: Indirect antibody detection ELISA

Purpose of Assay: Certified by the OIE in May 2008 as fit for serological diagnosis of type A avian influenza in chickens (specific to IgG in serum) and for the following purposes:

1. To demonstrate historical freedom from infection in a defined population (country/zone/compartment/flock);
2. To demonstrate re-establishment of freedom after outbreaks in a defined population (country/zone/compartment/flock);
3. To confirm diagnosis of suspect or clinical cases;
4. To estimate prevalence of infection to facilitate risk analysis in non-vaccinated populations (surveys/herd health schemes/disease control);
5. To determine immune status in individual animals or populations (post-vaccination)

Species and Specimen: Chicken serum

1. Information on the kit

Please refer to the kit insert available on the OIE Registry web page or contact manufacturer at:

BioChek B.V.
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2. Summary of validation studies

Analytical characteristics

Group specificity - A panel of sera representing all avian influenza (AI) haemagglutinin serotypes (H1 – H15) were tested. This panel represents antibody standards currently used by the VLA for serotyping strains of AI virus by haemagglutination inhibition (HI). All sera in this panel are also positive by agar gel immunodiffusion (AGID). These sera were derived from chickens experimentally infected with isolates from wild and

domestic birds with blood samples collected 14-21 days post exposure. All samples supplied by VLA Weybridge tested positive on the BioChek ELISA. These results confirm that the BioChek ELISA is capable of detecting group specific antibody across all the H-types of AI virus.

Analytical sensitivity - The BioChek ELISA was compared with two other commercial ELISA kits in the titration of a standard reference serum from VLA Weybridge. The BioChek ELISA demonstrated an endpoint titre of 1:64,000 compared to titers of 1:64,000 (Idexx ELISA) and 1:1,600 (KPL ELISA) for the other two kits.

Analytical specificity - Chicken antisera raised against other pathogens commonly present in poultry were tested in the ELISA. They included antisera to adenovirus, avian encephaIomyelitis, avian reovirus, E. coli, fowlpox, infectious bronchitis virus, infectious bursal disease, infectious laryngotracheitis, mycoplasma gallisepticum, mycoplasma synoviae, paramyxovirus 1, salmonella pullorum and turkey rhinotracheitis). Panels of monospecific hyperimmune sera derived from SPF chickens that had been either experimentally infected or vaccinated with the different pathogens were obtained from different suppliers. All samples tested negative on the BioChek ELISA.

Repeatability data - The Quality objectives of BioChek are to keep all %CVs below 20% and also to make sure that the overall mean of the control samples stays within 2SDs of the mean in every production run. Ranges are generated and must be adhered to; they are quoted in the summary table below raw data. When a new batch of control is produced it is run in Quality Control assays for 1 year alongside existing controls to generate ranges prior to introduction. Over a 2 year period, the diagnostic kits have been manufactured consistently and this has been confirmed by customer satisfaction with the reference control repeatability in many different laboratories.

Diagnostic Characteristics

Threshold determination

The BioChek (BC) AI ELISA cut off was determined by direct comparison to competitive ELISAs in the commercial market. Based on comparative endpoint titrations (see Analytical sensitivity, above), a cut off was selected that represented optimal analytical sensitivity. The cut off was set prior to specificity studies to ensure the analytical sensitivity was consistently maintained.

Diagnostic sensitivity (DSe) and specificity (DSp) estimates with 95% confidence limits (CI)

The following results document the fitness for purposes 1, 2, 3 and 4 mentioned above. The diagnostic sensitivity (DSe) was estimated at:

- Apparent diagnostic sensitivity of 100.00% (95% confidence interval: [91.19% to 100.00%], number of flocks tested: 2, number of chickens tested: 40, flock infection status determined using HI tests conducted at VLA) in clinically affected chickens (vaccinated for IBD NDV IBV REO and TRT) in Rawda/Saudi Arabia. The BC AI ELISA identified 40/40 sera as positive

compared to HI VLA in which 11 of 35 were positive (5 samples had insufficient sera) which suggests that the BC AI ELISA might be more sensitive than the HI.

- A further study, (number of flocks tested: 26, number of chicken tested: 253, flock infection status determined clinically and using an HI test specific for H9) in non-vaccinated chickens of different breeds and ages in South Korea, showed a higher detection rate of the BC AI ELISA (185/253) compared to HI H9 (137/253). These results again suggest that the BC AI ELISA might be more sensitive. The sensitivity of the BC AI ELISA in detecting infected flocks was estimated at 96.15% (25/26) in the same study.

The following results document the fitness for purposes 1, 2 and 4 mentioned above. The diagnostic specificity (DSp) was estimated at:

- 100.00% (95% confidence interval: [90.26% to 100.00%], number of flocks tested: 2, number of chickens tested: 200 but only 36 samples from a pool of 100 samples from each flock were tested) in SPF chickens from Germany,

- 100.00% (95% confidence interval: [96.45% to 100.00%], number of flocks tested: 1, number of chickens tested: 102, true status determined historically/clinically) in chickens (vaccinated against NDV IB, IBD, TRT, REO and CAV) from Scotland,

- 99.23% (95% confidence interval: [98.72% to 99.58%], number of flocks tested: 76, number of chickens tested: 1825, true status determined historically/clinically) in chicken (vaccinated against IBD, IBV, REO, etc.) from broad geographical and age spread, and

- 99.61% (95% confidence interval: [97.83% to 99.99%], number of chickens tested: 254, true status determined after Deventer AHS HI screening for all 16 H-types) in broiler and layer chickens (vaccinated against IBD, NDV, IB, REO, CAV and TRT) from the Netherlands.

The following results document the fitness for purposes 5.

- One hundred and eleven (111) independent samples collected randomly from 3 flocks (group 1, 2 and 3) of H5N2-vaccinated chickens (origin: Holland) were tested 2, 3, 4, 5 weeks post vaccination and the sensitivity in detecting a vaccination response was estimated 85.71% (24/28, 95% confidence interval: [67.33% to 95.97%]) in week 2 p.v. and 100.00% (83/83, 95% confidence interval: [95.65% to 100.00%]) in all other weeks tested. One hundred and seven (107) independent samples collected randomly from 3 flocks (group 4, 5 and 6) of H5N6-vaccinated chickens (origin: Holland) were tested 2, 3, 4, 5 weeks post vaccination and the sensitivity in detecting a vaccination response was estimated 88.89% (24/27, 95% confidence interval: [70.84% to 97.65%]) in week 2 p.v. and 98.75% (79/80, 95% confidence interval: [93.23% to 99.97%]) in all other weeks tested. All samples from control birds tested negative in both the HI and the BC AI ELISA (64 samples).

- Twelve (12) birds vaccinated with AIV H5N2 MSV+5 were tested 3, 4, 5 weeks post vaccination by Intervet HI and BioChek ELISA for comparison. Of 36 samples tested; 32 were positive by the HI test and 4 negative. The sensitivity in detecting a vaccination response was estimated at 31/32: 96.88%, 95% confidence interval: (83.78% to 99.92%) by the BioChek ELISA kit relative to the HI test.

- Birds experimentally infected or vaccinated [1) 50 wk old SPF layers 28 days p.i. with LP H7N1; 2) 4 wk old SPF layers 10 days p.i. with LP H7N1; 3) 50 wk old SPF layers; 4) 4 wk old SPF broilers 28 days p.v. with inactivated Nobilis H5N2 vaccine; 5) 4 wk old SPF broilers 28 days p.i. with H9N2; 6) 4 wk old SPF broilers 28 days p.i. with LP H5N2: 7) 4 wk old SPF layers 10 days p.i. with LP H5N2 and 8) 50 wk old SPF layers 28 days p.i. with H6N2] were tested by AHS Deventer (ring trial for avian influenza antibody detection in serum [2006]) using AGID, HI

and BioChek ELISA. BioChek ELISA showed a good level of sensitivity compared to the two others tests in this trial:

	Biochek	AGID	HI (H5 specific)
Group 1	100% POS	91% POS	-
Group 2	100% POS	94% POS	-
Group 3	100% NEG	94% NEG	100% NEG
Group 4	89% POS	35% POS	100% POS
Group 5	100% POS	100% POS	-
Group 6	78% POS	26% POS	93% POS
Group 7	83% POS	53% POS	59% POS
Group 8	89% POS	82% POS	-

Agreement between tests

For this calculation, the results of the following studies have been used:

1. Vaccination trials – Biochek + HI AIV H5 tests compared, 218 samples from vaccinated chickens tested + 64 negative control samples.
2. Comparison of BioChek with HI test on Intervet Vaccinated flocks, H5N1, 36 samples from vaccinated chickens tested + 8 negative control samples.
3. Field samples, Deventer AHS HI screening for all 16 haemagglutinins, 254 samples tested.

		HI test		
		Reactor	Non-Reactor	Totals
BioChek ELISA	Reactor	241	1	242
	Non-Reactor	9	329	334
	Totals	250	330	580

Relative Diagnostic Sensitivity (BioChek relative to HI): 241/250 or 96.40%, 95% confidence interval: (93.28% to 98.34%)
Relative Diagnostic Specificity ((BioChek relative to HI): 325/326 or 99.69%, 95% confidence interval: (98.30% to 99.99%)
Apparent prevalence HI test: 250/576 or 0.43
Apparent prevalence BioChek test: 242/576 or 0.42

Agreement can be quantified using the kappa statistic:

Observed proportion agreement	(241 + 325)/576	=	0.983
Chance proportion agreement (both +)	0.43 x 0.42	=	0.181
Chance proportion agreement (both -)	0.57 x 0.58	=	0.331
Chance proportion agreement	0.181 + 0.331	=	0.512
Observed minus chance agreement	0.984 – 0.512	=	0.471
Maximum possible agreement beyond chance	1 – 0.512	=	0.488
Kappa	0.471/0.488	=	0.965

This shows a strong kappa value close to 1.0 and therefore high degree of agreement between tests.

Reproducibility

A first international ring trial for avian influenza antibody detection in serum was conducted in 2006 with experimentally infected and vaccinated birds (126 samples from SPF layers and broilers, 4 or 50 weeks old and infected with AIV [either LP H7N1, H9N2, LP H5N2,or H6N2] or vaccinated with inactivated Nobilis H5N2 vaccine). The samples were prepared at the Animal Health Service of Deventer in the Netherlands and sent to 49 laboratories worldwide to be tested by ELISA (including BioChek ELISA), AGID, and HI. The results were collected and summarized by the Animal Health Service.

The BioChek ELISA was used by 9 laboratories on 8 duplicated samples (7 positives + 1 negative). Seven out of the 9 laboratories demonstrated reproducible results and identified all samples correctly. The two other laboratories experienced problems in correctly identifying some of the positive samples. Overall all, reproducibility was considered to be acceptable.

Application

This is an ongoing process. Testing laboratories should participate in proficiency testing and laboratory training programmes organized by OIE Reference Laboratories.

References

Chapter 2.3.4., Avian Influenza, *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals,* 2008, OIE, pp. 465-481.