OIE Procedure for Registration of Diagnostic Kits

Abstract sheet

Name of the diagnostic kit: Newcastle Disease Virus antibody Test Kit ELISA
Manufacturer: BioChek UK Ltd
OIE Approval number: 20140109
Date of Registration: May 2014

Disease: Newcastle Disease

Pathogen Agent: Newcastle Disease Virus (NDV)

Type of Assay: Indirect antibody detection ELISA

Purpose of Assay: Certified by the OIE in May 2014 as fit for detecting Newcastle disease virus specific IgG antibodies in chicken sera and for the following purposes:

1. To demonstrate historical freedom from infection in a defined population (country/zone/compartment/flock);
2. To determine immune status in individual animals or populations (post-vaccination);
3. To monitor infection or disease in unvaccinated populations;
4. To estimate prevalence of infection to facilitate risk analysis in non-vaccinated populations (surveys/flock health schemes/disease control).

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.
Burg Bracklaan 57
2811 BP Reeuwijk
Holland
E-mail: info@biochek.com
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2. Summary of validation studies

Analytical characteristics

Analytical sensitivity

1. Biochek test results for experimentally infected chickens that were sampled 7-14 days after vaccination/exposure (test carried out by Animal Health Service Ltd)

Description of samples:

1. Clone 30 Vaccination, Pooled sample taken 7 days post vaccination.
2. Clone 30 Vaccination, Pooled sample taken 14 days post vaccination.
3. NDW Ulster Vaccination, Pooled sample taken 10 days post vaccination.
4. SPF serum (1year old layers, Pooled sample)
5. Avinew VG/GA Vaccination, Pooled sample taken 7 days post vaccination.
6. Avinew VG/GA Vaccination, Pooled sample taken 10 days post vaccination.
7. AVIPRO ND HB1 Vaccination, Pooled sample taken 10 days post vaccination.

sample no	1	2	3	4	5	6	7
Haemagglutination inhibition (HI)	pos	pos	pos	neg	pos	pos	pos
Biochek ELISA	pos	pos	pos	neg	**pos/neg**	pos	pos

2. A total of 11 samples serially diluted from highly positive sera 3 x vaccinated Broiler Breeders (Netherlands origin) taken at 26wks of age and tested on Biochek ELISA and HI using Scitech Laboratories for the comparison.

96 experimentally diluted samples tested in all on both HI and ELISA

Summary of results table:

	ELISA POS	ELISA NEG	totals
HI POS	83	3	86
HI NEG	1	9	10
totals	84	12	96

Analytical specificity

From populations of SPF chicken flocks which have been either experimentally infected or vaccinated (hyperimmune) with the relevant chicken pathogens listed in the table below:

Infectious Bronchitis virus (IBV) 4/91DEV deventer institute Holland	IBV 4/91DEV deventer institute Holland	IBV 4/91INT Intervet Holland Avian
IBV 793BVLA VLA weybridge	Fowl Adenovirus Lohmann animal health	IBV D1466 deventer institute Holland
IBV CR88 Merial France	IBV CR98 Merial France	IBV D274INT Intervet Holland
IBV D1466INT Intervet Holland	IBV D274 deventer institute Holland	EColi 1 Lohmann animal health
IBV D3128 deventer institute Holland	IBV D8880 deventer institute Holland	Fowlpox deventer institute Holland
Salmonella pullorum	EColi 2 Lohmann	ILT AGP deventer institute

IFAH Compton	animal health	Holland
IBD deventer institute Holland	Infectious laryngotracheitis (ILT)deventer institute Holland	Mycoplasma gallisepticum deventer institute Holland
IBV M41 deventer institute Holland	IBV M41INT Intervet Holland	Paramyxovirus 3 deventer institute Holland
Mycoplasma synoviae deventer institute Holland	Paramyxovirus1 (NDV LaSota) deventer institute Holland	Avian Rhinotracheitis strain A Intervet Holland
Avian REOvirus 1133 Intervet Holland	Avian REOvirus 2534 Intervet Holland	Encephalomyelitis deventer institute Holland
Avian Rhinotracheitis strain C Intervet Holland	Influenza AGP Influenza H5 Influenza H7	

The Newcastle Disease Virus antibody Test Kit ELISA test negative for all samples except NDV positive samples

Repeatability data:

Repeatability testing Biochek NDV ELISA summary					
Within runs data 4 assays 1 operator					
	Mean	SD	%CV	No of run	No of sample
Low control	2516	60	2	4	4
Medium Control	4232	163	4	4	4
High Control	12128	508	4	4	4
Between runs data 24 assays 2 operators					
	Mean	SD	%CV	No of run	No of sample
Low control	2444	168	7	24	4
Medium Control	4244	233	5	24	4
High Control	11509	668	6	24	4

Conclusion: All %CV are below 10%.

<u>Diagnostic Characteristics</u>

Threshold determination

The Biochek Newcastle Disease Virus antibody Test Kit ELISA cut off was determined by comparison to leading market competitor and Gold standard HI to establish initial cut off of 0.35 S/P 1159 titre for positive sera.

Negative populations summary (Positive titers at =>1159)						
Flock name	No Samples	Mean S/P	SD	Median	Highest	Lowest
SPF sera	36	130	69	106	325	70
Field Negs	72	173	216	149	1907	17
AHS SPF	79	201	201	119	1073	7
AHS Negs	167	246	271	119	1132	7
Totals/averages	354	187	189	123	1109	25

One sample tested positive using a cut off of 0.35 giving 99.7% specificity

Positive sera AHS trial of animals sampled 7d-14days post vaccination approximately when IgG becomes detectable (Positive titers at =>1159)

Description of samples:	Biochek titre
1. Clone 30 Vaccination, Pooled sample taken 7 days post vaccination.	2048
2. Clone 30 Vaccination, Pooled sample taken 14 days post vaccination.	16384
3. NDW Ulster Vaccination, Pooled sample taken 10 days post vaccination.	2195
4. SPF serum (1year old layers, Pooled sample)	0
5. Avinew VG/GA Vaccination, Pooled sample taken 7 days post vaccination.	1261
6. Avinew VG/GA Vaccination, Pooled sample taken 10 days post vaccination.	5793
7. AVIPRO ND HB1 Vaccination, Pooled sample taken 10 days post vaccination.	4390

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

The diagnostic specificity (DSp) was estimated at:

1. Field samples

79 samples from SPF female layers (leghorns 60 weeks old) samples provided by the Animal Health Centre in Deventer Holland

2. Field samples

Field samples from Dutch flocks (167 in total) tested at AHS Deventer Holland from various Broiler flocks ranging in age from 38D to 42D sampled at slaughter and a breeder flock 24weeks old female breeders. All were tested negative HI and compared to Biochek ELISA.

3. Field samples

A total of 516 samples were collected ranging from 07W to 68W of age from SPF Leghorn flocks of German origin. These were tested on HI and compared to the Biochek ELISA.

	Negative Reference Samples
ELISA Positive	9
ELISA Negative	753

The diagnostic specificity was estimated with these samples at 98.8 %

The diagnostic sensitivity (DSe) was estimated at:

Experimental samples: 480 samples from Broiler flocks at slaughter vaccinated 01D and 21D with live Ulster strain vaccine.

	Positive Experimental Reference Samples
ELISA Positive	480
ELISA Negative	0

The diagnostic sensitivity was estimated with these samples at: 100%

Agreement between tests (with the haemaglutination test)

For the vaccinated reference animals, the results of the following studies have been used: Samples from broilers of various ages with known HI titers 480 samples

For the uninfected reference animals, the results of the following studies have been used: Field samples negative (see above).

	HI test		
Biochek ELISA	Reactor	Non-Reactor	Totals
Reactor	483	6	489
Non-Reactor	3	750	753
Totals	486	756	1242

Relative Diagnostic Sensitivity (Biochek relative to HI) = 483/486 = 99.4%
Relative Diagnostic Specificity ((Biochek relative to HI) = 750/756 = 99.2%
Apparent Prevalence HI = 483/1242 = 0.389
Apparent Prevalence Biochek ELISA = 486/1242 = 0.391

Agreement can be quantified using the kappa statistic:

Observed proportion agreement	(483 + 750)/1242	= 0.993
Chance proportion agreement (both +)	0.389 x 0.391	= 0.152
Chance proportion agreement (both -)	0.152x 0.152	= 0.023
Chance proportion agreement	0.152 + 0.023	= 0.175
Observed minus chance agreement	0.993 – 0.175	= 0.818
Maximum possible agreement beyond chance	1 – 0.175	= 0.825
Kappa	0.818/0.825	= 0.992

This shows good kappa value close to 1.0 and therefore in this study a good degree of agreement between tests.

Reproducibility

A Newcastle Disease Virus Antibody ring trial was organised by the R&D Laboratory of the Dutch Animal Health Service, Deventer, the Netherlands.

In total, 120 different laboratories participated from 37 countries from Africa, Asia, Europe, and South America in this trial.

The NDV Antibody Ring Trial consisted of 8 freeze-dried sera, that were sent to each of the participants with the request to test them for antibodies against NDV using all the techniques in operation at the time (in particular Biochek Newcastle Disease Virus antibody Test Kit ELISA and Haemaglutination Inhibition Test).

For the Biochek ELISA, 25 laboratories used the kit.

Sample ID	Origin:
1	Clone 30 Vaccination, Pooled sample taken 7 days post vaccination.
2	Clone 30 Vaccination, Pooled sample taken 14 days post vaccination.
3	NDW Ulster Vaccination, Pooled sample taken 10 days post vaccination.
4	SPF serum (1year old layers, Pooled sample)
5	Avinew VG/GA Vaccination, Pooled sample taken 7 days post vaccination.
6	Avinew VG/GA Vaccination, Pooled sample taken 10 days post vaccination.
7	Vaccinated and challenged (vvIBDV) chickens
8	AVIPRO ND HB1 Vaccination, Pooled sample taken 10 days post vaccination.

Using the Biochek ELISA, 23 of the 25 laboratories scored the SPF sample negative (sample 4). One laboratory scored it positive in duplicate, and another laboratory reported a negative and positive result.

All laboratories scored positive results with samples 2, 6, 7 and 8.

23 laboratories scored positive results, and 2 laboratories a negative and a positive result with sample 3.

22 laboratories reported positive results, 1 laboratory negative results and 2 laboratories a negative eand a positive result with sample 1.

13 laboratories reported positive results, 8 laboratories negative results and 4 laboratories a negative and a positive result with sample 5.

Application

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

References

Chapter 2.3.14., Newcastle disease, *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals,* 2012, OIE, pp. 555-573.