Newcastle Disease Antibody Test Kit (NDV)

BioChek
SMART VETERINARY DIAGNOSTICS

BioChek Poultry Immunoassays

Product Number CK 116

Description of Test

The NDV ELISA kit will measure the amount of antibody to NDV in the serum of chickens. Microtitre plates have been pre-coated with inactivated NDV antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-NDV antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-NDV antibodies bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-NDV antibody is present and the intensity is related to the amount of anti-NDV antibody present in the sample.

The validation data for this kit have been certified by the OIE, based on expert review, as fit for the following purposes:
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).

(As stated in the resolution adopted by the OIE World Assembly of Delegates).

Reagents provided:

1. **NDV Coated plates**. Inactivated viral antigen on microtitre plates.
2. **Conjugate reagent**. Anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v).
3. **Substrate tablets**. pNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate reagent**. Diethanolamine buffer with enzyme co-factors.
5. **Stop solution**. Sodium Hydroxide in Diethanolamine buffer.
6. **Sample diluent reagent**. Phosphate buffer with protein stabilisers and sodium azide preservative (0.2% w/v).
7. **Wash buffer sachets**. Powdered Phosphate Buffered Saline with Tween.
8. **Negative control**. Specific Pathogen Free serum in Phosphate buffer with protein stabilisers and sodium azide preservative (0.2% w/v).
9. **Positive control**. Antibodies specific to NDV in Phosphate buffer with protein stabilisers and sodium azide preservative (0.2% w/v).

Materials and Equipment required (not provided with kit):

Precision Pipettes and disposable tips
8 or 12 channel pipette/repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions:

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation:

1. **Substrate Reagent**. To make substrate reagent, add 1 tablet to 5.5 ml of substrate buffer and allow to mix until fully dissolved (approx. 10 minutes). The prepared reagent should be made on day of use but will be stable for one week if kept in dark at +4 °C. Drop tablets into clean container and add appropriate volume of substrate buffer.
   DO NOT HANDLE TABLETS WITH BARE FINGERS
2. **Wash Buffer**. Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing.
3. All other kit components are ready to use but allow them to come to room temperature (22-27°C) before use.

Sample preparation:

1. Dilute each test sample 1:500 in sample diluent reagent.

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING.

Test procedure:

1. Remove NDV coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1.
3. Add 100 µl of positive control into wells C1 and D1.
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (350µl per well). Invert plate and tap firmly on absorbent paper until no moisture is visible.
6. Add 100 µl of Conjugate reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the assay to be valid the mean negative control absorbance should read below 0.30 and the difference between the mean negative control and the mean positive control should be greater than 0.15.

The NDV positive control has been carefully standardised to represent significant amounts of antibody to NDV in chicken serum. The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio).

Interpretation of results:

Samples with an S/P of 0.35 or greater contain anti- NDV antibodies and are considered POSITIVE.

1. Calculation of S/P ratio:

$$\frac{\text{Mean of Test Sample - Mean of negative control}}{\text{Mean of Positive control - Mean of negative control}} = \text{S/P}$$

2. Calculation of Antibody Titre:

The following equation relates the S/P of a sample at a 1:500 dilution to a titre.

Log10 Titre = 1.0 * Log (SP) + 3.52

Antilog = Titre

S/P value	**Titre Range**	**Antibody status**
0.349 or less	1158 or less	No antibody detected
0.350 or greater	1159 or greater	Positive

This test is highly specific for antibodies against Newcastle Disease Virus. However, be aware that false positive reactors can occur in rare circumstances. Therefore confirmation with an established reference method is required for a final diagnosis.

BioChek has a software program available which can be used with the NDV kit to calculate S/P values, titres and provide general flock profiling.

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Appendix to Newcastle Disease Antibody Test Kit (NDV) Kit Insert
Product Number: CK116
OIE Registration Number: 20140109

Summary of Validation Data

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	IBV D274INT Intervet Holland

	France	
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 IFAH Compton	EColi 2 Lohmann animal health	ILT AGP deventer institute 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%.

Diagnostic Characteristics

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.