BioChek
SMART VETERINARY DIAGNOSTICS

ILT

Data Pack

Infectious laryngotracheitis Antibody detection ELISA

(Detects antibodies to avian laryngotracheitis virus)

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SUMMARY

Kit

- 5 plates, strip plate format
- Indirect ELISA
- Run at room temperature
- Incubation times: 60-60-30
- Read at: 405nm
- 1:500 dilution

Key Performance Features

Sensitivity

Positive reactions 10 - 14 days after infection

Specificity

Highly specific test, both on monospecific antisera as on sera of 60 week SPF old leghorns, less than 3 % non-specific reactions.

Reproducibility

Plate CV's lower than 10%, lot to lot reproducibility less than 15% of standard kit.

Applications

Vaccination check

Test flock after vaccination in order to establish efficiency of vaccination. Answers to key questions like "did the vaccine actually stimulate the immune system" can be found by testing 2 - 5 weeks after vaccination. The ILT vaccines don't generate a very strong serological response.
Please keep in mind that the antibody response only indicates that the vaccination has challenged the immune system. Protection of the chicken is mediated primarily by the cellular immune response.

Field infection

About 10 - 20 days after infection seroconversion will show.

BioChek Poultry Immunoassays

Infectious Laryngotracheitis Antibody Test Kit (STRIP PLATE FORMAT)

Catalogue Code CK213

Description of Test

The ILT ELISA kit will measure the amount of antibody to ILT in the serum of chickens. Microtitre plates have been pre-coated with inactivated ILT antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-ILT 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-ILT antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow color is developed if anti-ILT antibody is present and the intensity is directly related to the amount of anti-ILT present in the sample.

Reagents provided

1. **ILT Coated plates**. Inactivated viral antigen on microtitre plates
2. **Conjugate reagent**. Sheep 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 buffer**. Diethanolamine buffer with enzyme co-factors
5. **Stop Solution**. Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent**. Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer**. Powdered Phosphate Buffered Saline with Tween
8. **Negative control**. Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control**. Antibodies specific to ILT in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or de-ionized 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 bio hazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidizing 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 for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 ℃.*

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 de-ionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at *+4 ℃.*

3. All other kit components are ready to use but allow to come to room temperature (22 - 27 °C) before use.

Sample preparation
Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:
1. Remove ILT 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 **60 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **60 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 **30 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 samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.15.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The ILT positive control has been carefully standardized to represent significant amounts of antibody to ILT 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 .2 or greater contain anti-ILT 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 samples at a 1 : 500 dilution to an end point titre

Log10 Titre = 1.1 * Log(SP) + 3.361

Antilog = Titre

S/P value	**Titre Range**	**Antibody status**
.500 or less	1070 or less	Negative
.501 or greater	1071 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

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

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DATA SHEETS

SPECIFICITY

Purpose
To determine the distribution and characteristics of chicken serum originating from SPF (Specific Pathogen Free) chickens, when tested on the BioChek ILT ELISA.

Procedure
91 samples from 50-90 week old SPF leghorns were obtained (Lohmann Cuxhaven Germany) and assayed using the standard protocol on the BioChek ILT ELISA

Results
The results are shown in Table 1 and Graph1

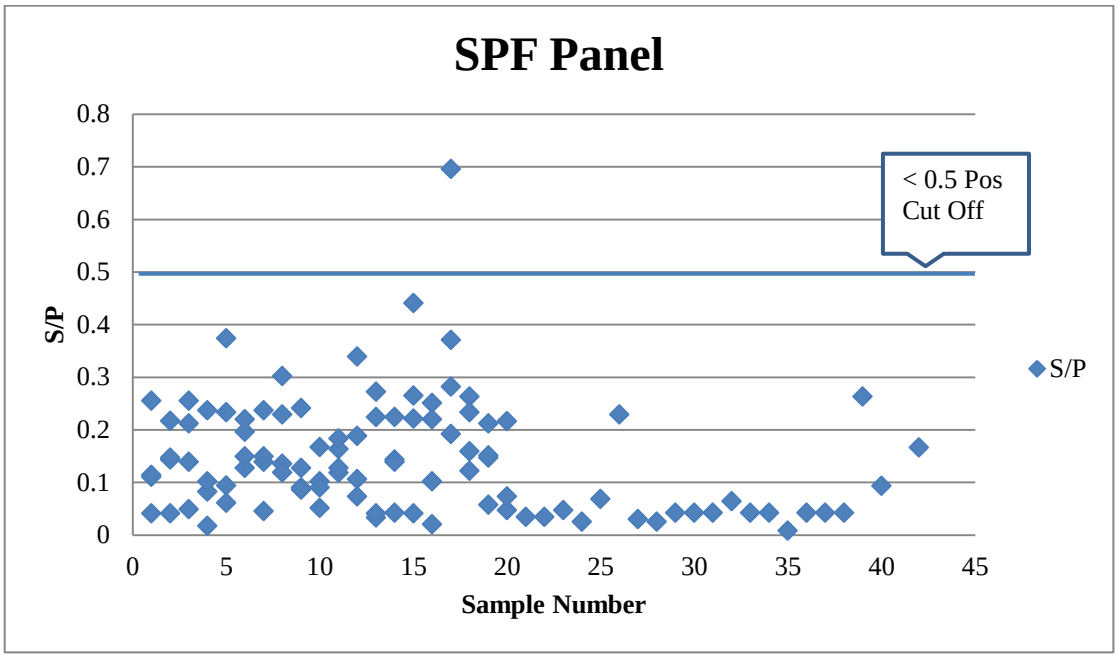
The data demonstrate that the BioChek ILT ELISA has 98% specificity on this sample panel. 99 out of 101 tested negative.

Table 1Specificity Panel

Cutoff	**Neg**	**S/P < .500**	
	Pos	**S/P > .499**	
test date	**aug/01**		
BioChek ILT	ELISA	FS 3674	
Samples SPF leghorns 50 - 90 weeks			
Summary of results:			
Samples tested: 101			
NEG	**99**	**Specificity:**	**98%**
POS	**2**		

sample No:	**S/P**	**result**	**sample No:**	**S/P**	**result**	**sample No:**	**S/P**	**result**
01	0.11	NEG -	01	0.255	NEG -	04	0.237	NEG -
02	0.041	NEG -	02	0.217	NEG -	05	0.061	NEG -
03	0.139	NEG -	03	0.255	NEG -	06	0.196	NEG -
04	0.082	NEG -	04	0.017	NEG -	07	0.045	NEG -
05	0.094	NEG -	05	0.374	NEG -	08	0.229	NEG -
06	0.127	NEG -	06	0.149	NEG -	09	0.241	NEG -
07	0.139	NEG -	07	0.149	NEG -	10	0.09	NEG -
08	0.135	NEG -	08	0.119	NEG -	11	0.127	NEG -
09	0.09	NEG -	09	0.127	NEG -	12	0.073	NEG -
10	0.102	NEG -	10	0.051	NEG -	13	0.041	NEG -
11	0.163	NEG -	11	0.183	NEG -	14	0.224	NEG -
12	0.188	NEG -	12	0.106	NEG -	15	0.265	NEG -
13	0.033	NEG -	13	0.272	NEG -	16	0.102	NEG -
14	0.143	NEG -	14	0.042	NEG -	17	0.192	NEG -
15	0.041	NEG -	15	0.221	NEG -	18	0.233	NEG -
16	0.02	NEG -	16	0.251	NEG -	19	0.151	NEG -
17	0.371	NEG -	17	0.696	**POS +**	20	0.073	NEG -
18	0.159	NEG -	18	0.263	NEG -	30	0.042	NEG -
19	0.147	NEG -	19	0.212	NEG -	31	0.042	NEG -
01	0.041	NEG -	20	0.047	NEG -	32	0.064	NEG -
02	0.143	NEG -	21	0.034	NEG -	33	0.042	NEG -
03	0.049	NEG -	22	0.034	NEG -	34	0.042	NEG -
04	0.102	NEG -	23	0.047	NEG -	35	0.008	NEG -
05	0.233	NEG -	24	0.025	NEG -	36	0.042	NEG -
06	0.22	NEG -	25	0.068	NEG -	37	0.042	NEG -
07	0.237	NEG -	26	0.229	NEG -	38	0.042	NEG -
08	0.302	NEG -	27	0.03	NEG -	39	0.263	NEG -
09	0.086	NEG -	28	0.025	NEG -	40	0.093	NEG -
10	0.167	NEG -	29	0.042	NEG -	41	0.59	**POS +**
11	0.118	NEG -	15	0.441	NEG -	42	0.166	NEG -
12	0.339	NEG -	16	0.22	NEG -	20	0.216	NEG -
13	0.224	NEG -	17	0.282	NEG -	01	0.114	NEG -
14	0.139	NEG -	18	0.122	NEG -	02	0.147	NEG -
			19	0.057	NEG -	03	0.212	NEG -

Graph 1 SpecificityPanel



DATA SHEETS

MONOSPECIFIC PANEL
Monospecific samples containing antibodies to various viruses.

Purpose
To determine if the BioChek ILT test kit cross-reacts with antibodies generated by other pathogens common in poultry flocks.

Procedure
A sample panel monospecific for antibodies of pathogens common in poultry was tested on the BioChek ILT ELISA.

Results / Conclusion
The results are shown in Table 2

The data demonstrates that only the monospecific serum sample for ILT tested positive on the BioChek ILT ELISA. This concludes that the test kit does not cross-react with antibodies directed at other avian pathogens

Table 2 Monospecific Panel

Test date: 29/08/2001
Assay : ILT **Lot No:** FS3674

Cutoff BC ILT:	NEG	S/P < .5			
	POS	S/P > .499			
Sample ID / Well			Raw O.D.	S/P Ratio	RESULT
ILT		**A02**	**1.631**	**4.3**	**POS +**
ILTAGP		**H02**	**1.116**	**2.772**	**POS +**
M41INT		B04	0.196	0.042	NEG -
M41		H01	0.299	0.347	NEG -
D3128		F01	0.283	0.3	NEG -
D1466		D01	0.229	0.139	NEG -
CR88		E03	0.163	0.03	NEG -
D274		E01	0.253	0.211	NEG -
D1466INT		F03	0.186	0.012	NEG -
D274INT		G03	0.152	0.03	NEG -
CR98		D04	0.144	0.03	NEG -
4/91INT		D03	0.145	0.03	NEG -
4/91DEV		C03	0.322	0.415	NEG -
793BVLA		B03	0.164	0.03	NEG -
PMV1		D02	0.198	0.047	NEG -
PMV3		G02	0.168	0.03	NEG -
REO1133		E02	0.192	0.03	NEG -
REO2534		F02	0.169	0.03	NEG -
IBD		C01	0.197	0.045	NEG -
Fpox		B01	0.208	0.077	NEG -
Adeno		A01	0.19	0.024	NEG -
TRTC		C04	0.155	0.03	NEG -
TRTA		E04	0.209	0.08	NEG -
Mg		B02	0.212	0.089	NEG -
Ms		C02	0.275	0.276	NEG -
AE		A03	0.19	0.024	NEG -
ECOLI2		A04	0.329	0.436	NEG -

DATA SHEETS

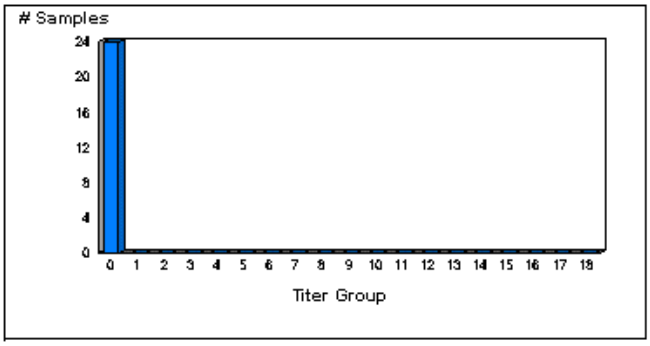
SENSITIVITY

Samples form commercial layers were taken before and after ILT vaccination. The vaccination has been done by eyedrop.

Flock:

Code :	00007
Reason for Testing:	PRE VACC
Bleed Date :	03/07/2001

Assay :	**ILT**	Dilution :	**500**
Bleeding Date :	**03/07/2001**	Samples :	**24**

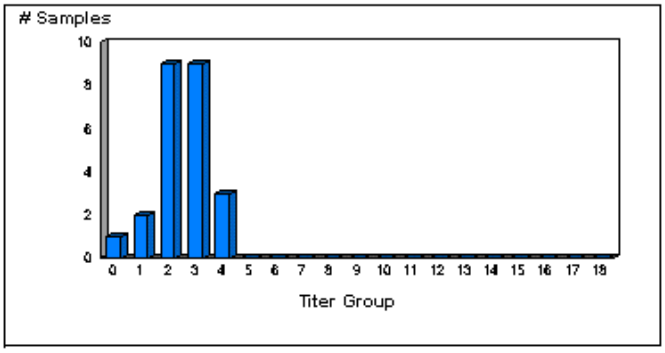


Mean Titer :	**60**
G.M.T. :	**50**
%CV :	**0**
Std.Dev. :	**38**

Flock:

Code :	00079
Age :	12W
Reason for Testing:	20D POST VACC
Bleed Date :	16/07/2001

Assay :	**ILT**	Dilution :	**500**
Bleeding Date :	**16/07/2001**	Samples :	**24**



Mean Titer :	**1115**
G.M.T. :	**925**
%CV :	**55**
Std.Dev. :	**618**

DATA SHEETS

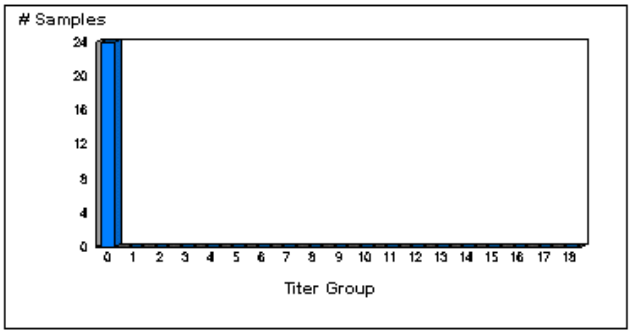
FIELD INFECTION

Clinical symptoms were presented in Broiler Breeders at 51/52 weeks. Samples were taken from at age 55 weeks.

Histogram prior to vaccination or infection, all samples test negative

Code :	00012
Age :	08W
House No. :	01
Reason for Testing:	ROUTINE NO VAC
Bleed Date :	03/07/2001

Assay :	**ILT**	Dilution :	**500**
Bleeding Date :	**03/07/2001**	Samples :	**24**

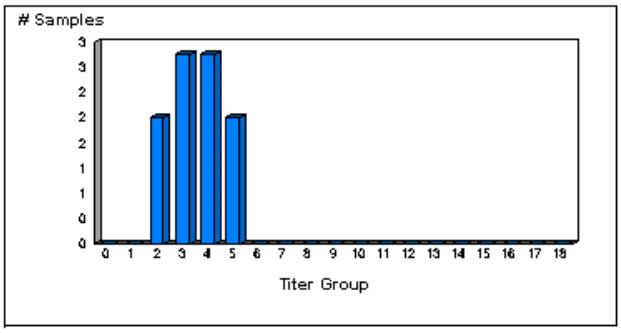


Mean Titer :	**162**
G.M.T. :	**70**
%CV :	**143**
Std.Dev. :	**232**

Histogram of samples taken 3 weeks after a field challenge, most or all samples test positive:

Code :	00094
Age :	55W
House No. :	05
Reason for Testing:	ILT FIELD CHALL
Type of Bird:	BB
Bleed Date :	08/10/2001

Assay :	**ILT**	Dilution :	**500**
Bleeding Date :	**08/10/2001**	Samples :	**10**



Mean Titer :	**2515**
G.M.T. :	**2254**
%CV :	**0**
Std.Dev. :	**1301**

DATA SHEETS

COMPARISON TO VN

The BioChek ILT ELISA test results are compared with VN results.
VN test done by Dr. Mizumura, Ghen Coorperation, Japan

Cutoff:	**BC ELISA S/P value**		**VN**	Date: Sept 2001
Negative	< .500		VN < 1	**Negative**
Positive	>=.500		VN >= 1	**Positive**
	Assay :	ILT		
	Lot No:	FS3674		

Sample ID	**S/P Ratio**	**Result BC**	**VN**	**Result VN**
01	0.67	POS +	1.25	POS +
02	0.30	NEG -	0.75	NEG -
03	0.48	**NEG -**	1	**POS +**
04	0.54	POS +	1.5	POS +
05	5.16	POS +	1.25	POS +
06	0.64	POS +	1.5	POS +
07	1.17	POS +	2	POS +
08	0.94	POS +	1.5	POS +
09	0.36	NEG -	0.75	NEG -
10	5.11	POS +	2.75	POS +
11	0.68	POS +	2.75	POS +
12	4.23	POS +	1.5	POS +
13	2.57	POS +	2	POS +
14	0.09	**NEG -**	**0.75**	**NEG -**
15	6.08	POS +	1.25	POS +
16	1.58	POS +	2	POS +
17	0.81	**POS +**	**0.75**	**NEG -**

Only sample 3 and sample 17 give conflicting results. For sample no 3 the BC ELISA test result is just below the cutoff with an S/P of .48, sample 17 gives a VN result just below the VN cutoff.

GENERAL INFORMATION ON ILT

Laryngotracheitis is a viral respiratory tract infection of chickens. The laryngotracheitis virus is classified as a member of the family Herpesviridae. Naturally occurring LTV strains vary in virulence from highly virulent strains causing high mortility and mortality and strains of low virulence that produce mild to inapparent infection.

Pathogenesis and Epizootiology

The chicken is the primary natural host of LTV. Although the disease affect all ages, the most characteristic signs are observed in adult birds.

Clinical signs

Severe epizootic form

Clinical signs appear 6 - 12 days following natural exposure. High morbidity (90 - 100%) and variable mortality (5% - 70%) average 10% - 20%.
Acute respiratory disease in chickens. Characteristic clinical signs include nasal discharge and moist rales followed by coughing and gasping. Marked dispnea and expectoration of bloodstained mucus is characteristic of severe epizootic forms of the disease.

Mild enzootic forms

In recent years mild enzootic forms of LT with low mortality (.1 - 2%) have been observed in intensive poultry producing areas. Clinical signs associated with mild enzootic forms include thriftiness, reduction in egg production, watery eyes, conjunctivitis, swelling of infraorbital sinuses, persistent nasal discharge and heamorrhagic conjunctivitis.
Most chickens recover in 10 - 14 days

Immunity

The principal mediator of LT resistance is the local cell-mediated immune response in the trachea.
Maternal antibodies do not protect to infection and does not interfere with vaccination.
Protection due to vaccination starts at about 8 days post vaccination and last until about 15 - 20 weeks after vaccination.

Diagnosis

In general LT diagnosis requires laboratory assistance as other respiratory pathogens can cause similar signs and lesions. Only in cases of severe acute disease with high mortality and expectoration of blood can LT be diagnosed on the basis of clinical signs.

Serology

There is only 1 serotype of the virus.