



ART

Data Pack

Avian rhinotracheitis (ART/TRT) Antibody ELISA
(Detects antibodies to avian pneumovirus)

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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

Detects antibodies to A and B strains for Avian pneumovirus.
SPF chickens 100% specificity

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 3 - 5 weeks after vaccination. See our interpretation manual for details on expected response to vaccination.

Field infection

About 10 - 20 days after infection seroconversion will show.

BioChek Poultry Immunoassays

Avian Rhinotracheitis Antibody Test Kit

For the detection of antibody to Avian Pneumovirus in Chickens and Turkeys

Catalogue Code CK120

Description of Test

The ART ELISA kit will measure the amount of antibody to ART in the serum of chickens and Turkeys. Microtitre plates have been pre-coated with inactivated ART antigen. Serum samples are diluted and added to the microtitre wells where any anti-ART 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-ART antibodies originally 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-ART antibody is present and the intensity is directly related to the amount of anti-ART present in the sample.

Reagents provided

1. **ART Coated plates.** Inactivated viral antigen(Avian pneumovirus)-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 ART 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 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 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 °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. Wash buffer will remain stable for use for 1 month if stored at +4 °C.
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 ART 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 the 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 ART positive control has been carefully standardised to represent significant amounts of antibody to ART in Chicken or Turkey 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-ART antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

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

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

$$\text{Log}_{10} \text{Titre} = 1.0 (\log_{10} \text{S/P}) + 3.52$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.349 or less	1158 or less	Negative
.350	1159 - 1655	Suspect
.500 or greater	1656 or greater	Positive

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

BioChek has available a software programme which can be used with the ART 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 ART ELISA.

Procedure

Samples from 60 week old SPF Leghorns were obtained and assayed using the standard protocol for the BioChek ART ELISA

Results/Conclusion

The results are shown in Table 1.

The results have been plotted on Graph 1 showing S/P value against sample number.

The data demonstrates that the BioChek AI ELISA has 100% specificity on this sample panel.

Table 1 ART SPF Panel

BioChek						
Weth. Venteweg 143, 2805 JN Gouda, NL						
Tel: +31 182 582 592 fax: +31 182 599360						
					Page :	1 Date : 05-11-1999
Interpretation of results			s/p	titre		
neg			<.250	<827		
Pos			>.350	>1158		
Name :	SPF SERA					
Reason :	ART SPECIFICITY					
Assay :	BioChek ART					
Mean Titer:	404					
G.M.T.:	371					
Well		Raw O.D.	S/P Ratio	Titer	Result	
-	A01	0.124				
-	A02	0.124				
+	A03	0.464				
+	A04	0.464				
1	B12	0.178	0.159	526	Neg	
2	C01	0.159	0.103	341	Neg	
3	C02	0.147	0.068	225	Neg	
4	C03	0.159	0.103	341	Neg	
5	C04	0.173	0.144	477	Neg	
6	B08	0.184	0.176	583	Neg	
7	B09	0.176	0.153	507	Neg	
8	B10	0.157	0.097	321	Neg	
9	B11	0.165	0.121	401	Neg	
10	C05	0.144	0.059	195	Neg	
11	C06	0.167	0.126	417	Neg	
12	C07	0.138	0.041	136	Neg	
13	C08	0.161	0.109	361	Neg	
14	C09	0.189	0.191	632	Neg	
15	C10	0.197	0.215	712	Neg	
16	C11	0.153	0.085	281	Neg	

Graph 1SPF Negative Panel

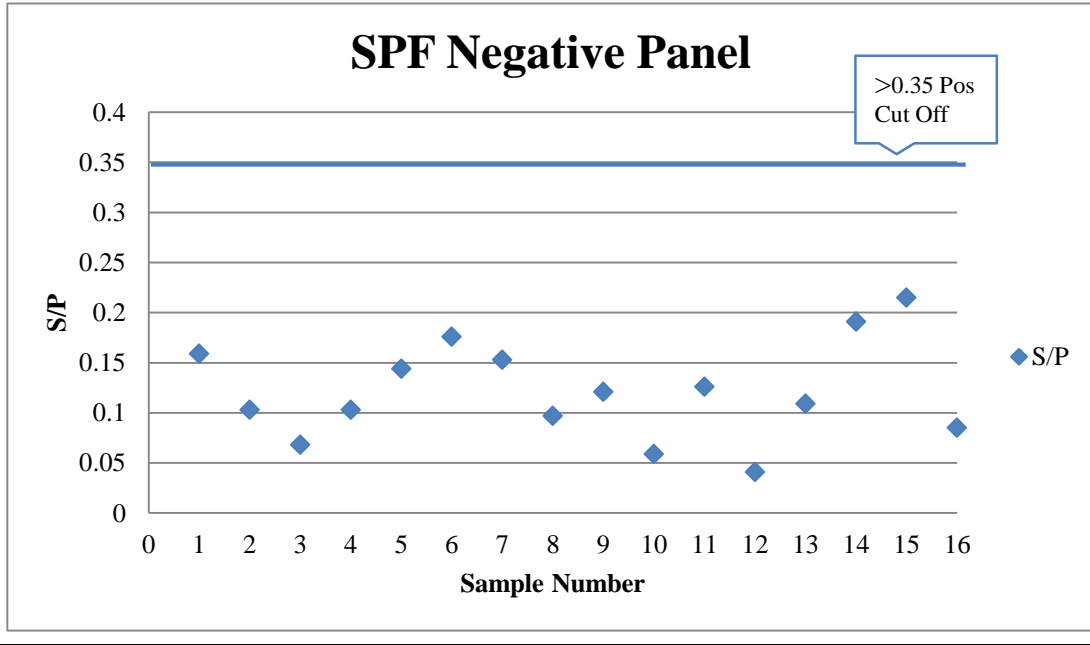


Table 3 Strain B
Report: BlockDiagram
Flock:

Name : STRAIN B
 Company : VACCINE
 Code : LIVE VACCINE
 Age : 14D
 Reason : 14D POST VAC
 Type : SP
 BleedDate : 29/04/1998

Assay : ART **Bleeding Date :** 29/04/1998
Mean Titer: 5 143 **Dilution :** 500
G.M.T.: 4671 **%CV :** 41

Well	Raw O.D.	S/P Ratio	Titer	Titergroup
- A01	0.154	0.00		
- B01	0.137	0.00		
+ C01	0.361	0.00		
+ D01	0.349	0.00		
1 F01	0.591	2.13	7 040	6
2 G01	0.697	2.63	8 715	7
3 H01	0.463	1.52	5 020	5
4 A02	0.323	0.85	2 805	3
5 B02	0.604	2.19	7 248	6
6 C02	0.356	1.00	3 328	4
7 D02	0.536	1.86	6 172	6
8 E02	0.447	1.44	4 765	5
9 F02	0.531	1.84	6 093	6
10 G02	0.266	0.57	1 904	2
11 H02	0.301	0.74	2 457	3
12 E01	0.536	1.86	6 172	6

DATA SHEETS

MONOSPECIFIC SAMPLE PANEL

Monospecific samples containing antibodies to various viruses.

Purpose

To determine if the BioChek ART 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 ART test.

Results / Conclusion

The results are shown in Table 1

The data demonstrates that all the monospecific serum samples for the various poultry pathogens tested negative on the BioChek ART ELISA. This concludes that the test kit does not cross-react with antibodies directed at other avian pathogens.

Table 1

Date : 05-11-1999
 Name : BioChek ART ELISA
 Reason : MONOSPECIFIC sample panel

Interpretation of results	s/p	titre
neg	<.350	<1158
Pos	>.500	>1656

Assay : ART

Dilution :500

sample	well	Raw O.D.	S/P Ratio	Titer	result
-	A01	0.124			
-	A02	0.124			
+	A03	0.464			
+	A04	0.464			
Adeno	A05	0.173	0.144	477	NEG
Fowlpox	A06	0.172	0.141	467	NEG
IBD	A07	0.141	0.05	166	NEG
IBV1	A08	0.18	0.165	546	NEG
ILT	B01	0.191	0.197	652	NEG
Mg	B02	0.181	0.168	556	NEG
Ms	B03	0.223	0.291	964	NEG
NDV	B04	0.175	0.15	497	NEG
Reo	B07	0.14	0.047	156	NEG
Reo	B05	0.173	0.144	477	NEG
PMV3	B06	0.17	0.135	447	NEG

Source of samples: Animal Health Service, Deventer, Holland