BioChek
SMART VETERINARY DIAGNOSTICS

MS

Data Pack

Mycoplasma synoviae Antibody Test Kit
(Detcts antibodies to mycoplasma synoviae bacteria)

CONTENTS

SUMMARY

Kit

- 5 plates
- Indirect ELISA
- Run at room temperature
- Incubation times: 30-30-15
- Read at: 405nm
- 1:500 dilution

Key Performance Features

Sensitivity

Ms similar to the common confirmation method for MS which is a serial dilution on the RPA (rapid plate antigen).

Specificity

All our mycoplasma tests are very specific. However we recommend confirming with other methods when finding positive results.

Reproducibility

Plate CV's lower than 10%.

Applications

Screening

Most common use of the BioChek Ms ELISA is screening flocks for positives. When positive samples are found confirm with alternative methods such as culture or PCR.
The Ms ELISA can also be used for confirmation of RSA positive flocks.

Vaccination monitoring

When using the BioChek Mycoplasma ELISA's for confirmation of success of vaccination one should first contact the vaccine manufacturer to get information on expected serology after vaccination.

BioChek Poultry Immunoassays
Mycoplasma synoviae Antibody Test Kit

Catalogue Code CK 115

Description of Test

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

Reagents provided:

1. **MS 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 buffer 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 (0.1% 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 (0.1% w/v).
9. **Positive control**. Antibodies specific to MS in Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% 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 - 6 ml of substrate buffer and allow to mix until fully dissolved (+/- 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

   POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING!!

Test procedure:

1. Remove MS 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 test result 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.

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 MS positive control has been carefully standardised to represent significant amounts of antibody to MS 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 0.5 or greater contain anti-Ms 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 an end point titre

Log10 Titre = 1.27 * (log10 S/P) + 3.156

Antilog = Titre

S/P value	Titre Range	Antibody status
0.499 or less	593 or less	Negative
0.500 or greater	594 or greater	Positive

For confirmation of status additional alternative testing should be performed.

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KI/CK115REV03

DATA SHEETS

SENSITIVITY

Purpose

To determine if BioChek MS antibody detection assay detects antibodies after active immunization/challenge .

Procedure

Antisera chickens (6) were infected with MS and MG at 3 weeks of age.
Serum samples were taken at 1, 2 and 6 weeks P.I. (post vaccination) and tested on RPA , HI , and the BioChek MS ELISA.
RPA and HI performed by PDRC Athens Georgia. At the end of the trial the samples were sent to BioChek UK Ltd for analysis on BioChek MS ELISA.

Results/Conclusion

See Table I
RPA tested positive 7 days post challenge
HI tests positive 14 days post challenge
BioChek Ms tests positive 21 Days post Challenge
Both RPA and the BioChek ELISA test negative on Mg challenged chickens.

In these trials , the BioChek MS antibody detection test has detected antibodies 21 D days post vaccination.

Table I Temporal Sensitivity of BioChek MS ELISA .

Mg callenged Birds

% positive

age	Days P.I	RPA Mg	RPA Ms	HI Mg	BC Ms
3W	D00	0	0	0	0
4W	D07	0	0	0	0
5W	D14	83	0	0	0
6W	D21	100	0	81	0

Ms Challenged Birds

% positive

age	Days P.I	RPA Mg	RPA Ms	HI Ms	BC Ms
3W	D00	0	0	0	0
4W	D07	0	16	0	0
5W	D14	0	100	50	0
6W	D21	0	100	100	50

Samples provided by Dr. S.H. Kleven PDRC Athens, GA in february 2005

RPA and HI performed by PDRC Athens Greorgia

BC (= BioChek) MS performed by BioChek UK Ltd.

DATA SHEETS

MONOSPECIFIC PANEL

Purpose

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

Procedure

A sample panel mono-specific for antibodies of pathogens common in Poultry, was tested on the BioChek MS test.

Results/Conclusion

Results are presented in Table II. The BioChek MS ELISA tested negative on all samples tested, with exception of the MS positive samples in the sample panel.

Only the mono-specific serum samples for MS tested positive on the BioChek MS ELISA. The conclusion is that the BioChek MS ELISA is specific to antibodies belonging to M. synoviae.

Table II Monospecific Panel

BioChek sample panel of sera antibody positive for antigens mentioned The BioChek Mg and the BioChek Ms assay test negative for all samples, except the homogolous sample(s)

Name : BC monospecific sample panel
Bleeding Date : 25-02-2002
Assay : Ms Lot: FS3713

Sample ID	S/P Ratio	RESULT			
4/91DEV	0.005	NEG	D274INT	0.024	NEG
4/91INT	0.024	NEG	D3128	0.024	NEG
793BVLA	0.024	NEG	D8880	0.026	NEG
adeno	0.024	NEG	ECOLI1	2.82	NEG
AE	0.024	NEG	ECOLI2	1.075	NEG
CR88	0.024	NEG	Fpox	0.024	NEG
CR98	0.024	NEG	IBD	0.024	NEG
D1466	0.174	NEG	ILT	0.024	NEG
D1466INT	0.024	NEG	ILTAGP	0.024	NEG
D274	0.024	NEG	M41	0.071	NEG
M41INT	0.024	NEG	REO1133	0.024	NEG
Mg	0.024	NEG	REO2534	0.024	NEG
Ms	**3.303**	**POS**	TRTA	0.024	NEG
PMV1	0.024	NEG	TRTC	0.024	NEG
PMV3	0.024	NEG			

Interpretation of results BioChek Ms ELISA
S/P=>.5 pos

E Coli samples are positive for Ms

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 MS ELISA.

Procedure

Samples from 36 week old Broiler Breeders were obtained and assayed using the standard protocol for the BioChek MS ELISA. These breeders have been vaccinated for Marek, Coccidioses, IBV, IBD, REO AE Fowl pox and Chicken Anemia.

The inactivated vaccines where given at 19 weeks of age.

Results / Conclusion

The results are shown the following report overleaf and Graph 1

The results have been plotted on Graph1 showing S/P value against sample number.

The positive cut off for the BioChek MS ELISA is an S/P value of 0.5. The data demonstrates that the BioChek MS ELISA has 100% specificity on this sample panel.

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Report: **Histogram/BlockDiagram** **Page :** 1 **Date :** 23-12-2005

Name : BOX 1
Company : NL
Age : 26W
Type : BB
Reason for Testing : SPECIFICITY

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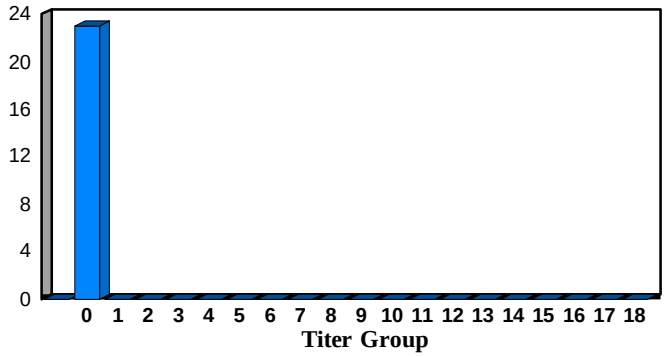
 # Samples



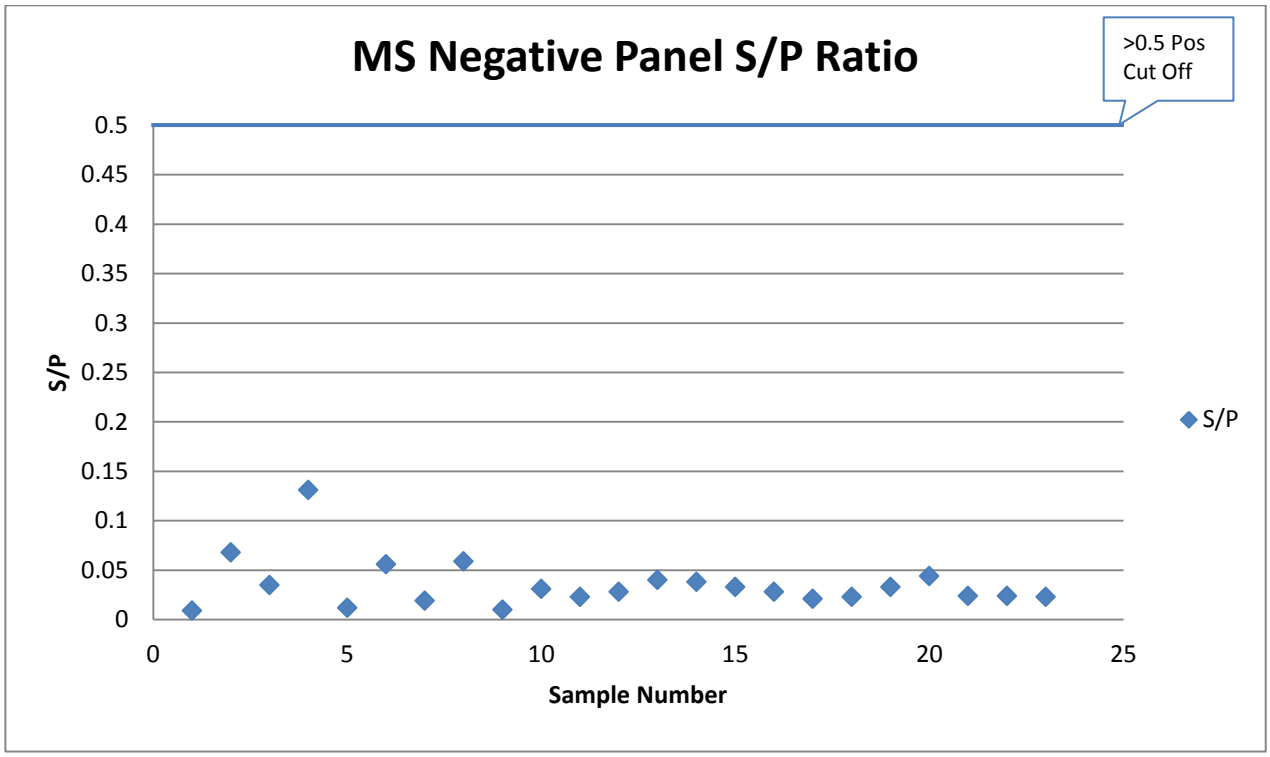
Assay : MS **Lot:** 1998
Bleeding Date : 05-08-1998 **Testing Date:** 23-12-2005

Mean Titer: 22
%CV : 99

Positive Cutoff S/P >= 0.50 **Total No. Samples:23** **Neg/Sus/Pos = 23 / 0 / 0**

Sample ID	Well	Raw O.D.	S/P Ratio	Titer	Titer Group	Result
-	A01	0.088	0.000			
-	A02	0.086	0.000			
+	A03	0.660	0.000			
+	A04	0.660	0.000			
01	A05	0.092	0.009	4		NEG -
02	A06	0.126	0.068	47		NEG -
03	A07	0.107	0.035	20		NEG -
04	A08	0.162	0.131	108		NEG -
05	A09	0.094	0.012	5		NEG -
06	A10	0.119	0.056	37		NEG -
07	A11	0.098	0.019	9		NEG -
08	A12	0.121	0.059	39		NEG -
09	B01	0.093	0.010	4		NEG -
10	B02	0.105	0.031	17		NEG -
11	B03	0.100	0.023	12		NEG -
12	B04	0.103	0.028	15		NEG -
13	B05	0.110	0.040	24		NEG -
14	B06	0.109	0.038	23		NEG -
15	B07	0.106	0.033	19		NEG -
16	B08	0.103	0.028	15		NEG -
17	B09	0.099	0.021	11		NEG -
18	B10	0.100	0.023	12		NEG -
19	B11	0.106	0.033	19		NEG -
20	B12	0.112	0.044	27		NEG -
21	C01	0.101	0.024	13		NEG -
22	C02	0.101	0.024	13		NEG -
23	C03	0.100	0.023	12		NEG -

Graph 1MS Specificity Panel



DATA SHEETS

REPRODUCIBILITY

Trial 1: Batch to batch reproducibility
Trial 2: Intra assay reproducibility

Trial 1: Batch to Batch Reproducibility

Purpose

In this trial a pre-diluted chicken serum sample RF06 containing a medium level of antibodies to MS was tested on several batches of the BioChek MS ELISA . The purpose of the trail is to assess batch to batch reproducibility.

Procedure

A known , pre-diluted MS sample, RF06 is assayed in duplicate on 7 different production batches of MS kits. Mean S/P values, standard deviation , and C.V. are calculated to asses the amount of variability between the different batches of kits.

Results/Conclusion

As can be seen in the corresponding table (table IV reproducibility) the batch variability on the 7 batches is as following:
For the RF06 sample results were:

Mean S/P	0.98
SD	0.08
%CV	8.57

These data demonstrate that there is limited variation (< 10%) , when comparing results from various production batches of the BioChek MS ELISA

IV Reproducibility of the BioChek MS ELISA **Date** **10-03-2006**

Sample High: prediluted serum RF06 containing positive titers of antibodies against MS
RF06 sample was tested on 7 different production batches

				Raw OD values controls + samples								
Name	batch no	manufacturing date	Assay	-	-	+	+	OD	OD	Mean S/P	No. Samples	**Result**
RF06	FS4205	07-04-2005	MS	0.120	0.125	0.886	0.929	0.862	0.866	0.9450	2	**Mean S/P** **0.98**
RF06	FS4247	23-06-2005	MS	0.111	0.113	0.743	0.744	0.691	0.663	0.8900	2	**SD** **0.08**
RF06	FS4263	03-08-2005	MS	0.157	0.121	0.872	0.880	0.764	0.811	0.8800	2	**%CV** **8.57**
RF06	FS4293	18-10-2005	MS	0.129	0.123	0.832	0.763	0.769	0.778	0.9650	2	
RF06	FS4313	23-11-2005	MS	0.148	0.146	0.827	0.788	0.884	0.860	1.1000	2	
RF06	FS4326	12-12-2005	MS	0.159	0.167	0.834	0.838	0.875	0.851	1.0400	2	
RF06	FS4355	10-03-2006	MS	0.156	0.151	0.864	0.858	0.903	0.897	1.0500	2	

Trial 2: Intra-Assay Reproducibility

Purpose

The purpose of the trail is to asses intra-plate reproducibility. The plate CV of the MS test kit should be less than 10%.

Procedure

A standard pre-diluted sample known positive for MS is assayed on 90 wells of a MS plate. MS test is run according to package insert.

Results/Conclusion

Results are shown in the table V.
The %CV of the sample (RF06) is 8.80 %.

Table V. Intra-plate Reproducibility of BioChek MS ELISA

BioChek Intraplate reproducibility study

Assay	**MS**
date	**13-12-2005**
Mean OD	**0.561**
st dev	**0.05**
%CV	**8.80**

Lot **FS4182**

	1	2	3	4	5	6	7	8	9	10	11	12
Row A	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06
OD	0.646	0.565	0.619	0.589	0.642	0.589	0.606	0.631	0.58	0.602	0.622	0.671
Row B	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06
OD	0.597	0.549	0.555	0.547	0.538	0.522	0.512	0.531	0.55	0.528	0.538	0.61
Row C	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	**NEG**
OD	0.56	0.491	0.537	0.514	0.542	0.507	0.523	0.503	0.517	0.525	0.535	0.104
Row D	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	**NEG**
OD	0.572	0.517	0.537	0.488	0.495	0.512	0.5	0.504	0.507	0.502	0.533	0.103
Row E	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	**POS**
OD	0.627	0.537	0.534	0.552	0.539	0.541	0.531	0.514	0.524	0.503	0.546	0.617
Row F	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	**POS**
OD	0.56	0.517	0.524	0.531	0.52	0.535	0.525	0.514	0.519	0.534	0.544	0.615
Row G	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	
OD	0.651	0.534	0.571	0.56	0.565	0.587	0.559	0.564	0.584	0.555	0.603	
Row H	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	
OD	0.68	0.618	0.681	0.617	0.642	0.617	0.599	0.653	0.604	0.65	0.66	