BioChek
SMART VETERINARY DIAGNOSTICS

MGMS

Data Pack

Mycoplasma gallisepticum/synoviae Combined Antibody Test Kit
(Detects antibodies to both mycoplasma gallisepticum and synoviae)

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

Mg: sensitivity similar to HI. 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 to confirm with other methods when finding postive results.

Reproducibility

Plate CV's lower than 10%.

Applications

Screening

The Most common use of the Ms/Mg ELISA is screening flocks for positives on the combination test. When positive samples are found, confirm with Ms or Mg ELISA. When these are also positive, confirm with another method.

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 gallisepticum/synoviae Antibody Test Kit (Mg/Ms)

Catalogue Code CK 215

Description of Test

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

Reagents provided:

1. **Mg/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 Mg/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 Mg/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 Mg/Ms positive control has been carefully standardised to represent significant amounts of antibody to Mg/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-Mg/Ms antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

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

Samples with an S/P of 0.499 or less are considered NEGATIVE.

Because the Mg/Ms combined test is a screening assay, it is not possible to generate antibody titre values that are valid for Mg or Ms.
The S/P generated for a sample will be for Mg or Ms. There is no differentiation between the two by this test.
For a general titre value, the following equation may be used:

Log10 Titre = 1.1 * Log (SP) + 3.156
Antilog = Titre

For identification of a positive sample, it will be necessary to run the sample again using a monospecific Mg kit (cat. No. CK114) or Ms (cat.no. CK115)

For confirmation of status additional alternative testing should be performed.

Distributor:
BioChek B.V.
Burg Bracklaan 57
2811 BP Reeuwijk
Holland
tel: +31 182 582 592
fax: +31 182 599 360
E-mail: info@biochek.com
Website: www.biochek.com

Manufacturer:
BioChek (UK) Ltd.
11 Mill farm business park
Millfield Road,Hounslow
London TW4 5PY

KI/CK215REV04

DATA SHEETS

SENSITIVITY

Purpose
To establish the time it takes for the BioChek MgMs antibody detection assay to detect antibodies after active immunization.

Procedure
6 antisera chickens were infected with Mg at 3 weeks of age and another 6 were infected with Ms at 3 weeks of age. Serum samples were collected every week.

Results/Conclusion
The results are shown in Table 1 and Table 2.

Mg positive samples
RPA tests positive 14 days post challenge
HI tests positive 21 days post challenge
BioChek MgMs tests positive 21 Days post Challenge
BioChek MgMsHS tests positive 14 Days post Challenge

Ms positive samples
RPA tests positive 7 days post challenge
HI tests positive 14 days post challenge
BioChek MgMs tests positive 21 Days post Challenge
BioChek MgMsHS tests positive 14 Days post Challenge

Table 1Sensitivity

Date: 27 september 2005

Mg temporal samples
% positive

age	Days P.I	RPA Mg	RPA Ms	HI Mg	BC MgMs	BC MgMsHS
3W	D00	0	0	0	0	0
4W	D07	0	0	0	0	0
5W	D14	83	0	0	0	17
6W	D21	100	0	81	17	67

Table 2
Ms temporal samples
% positive

age	Days P.I	RPA Mg	RPA Ms	HI Ms	BC MgMs	BC MgMsHS
3W	D00	0	0	0	0	0
4W	D07	0	16	0	0	0
5W	D14	0	100	50	0	50
6W	D21	0	100	100	17	67

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 MgMs ELISA.

Procedure

62 SPF birds at one day old and 43 birds at 24 and 33 weeks were obtained and assayed using the standard protocol for the MgMs ELISA.

Results

The results are shown in Table 2 and Table 3

The data demonstrates that the BioChek MgMs ELISA has 100% specificity on these sample panels.

Table 2 Specificity Panel

BioChek								
Page : 1				Date : 09-06-2008				
Report:		BlockDiagram						
Name :		01D						
Age :		01D						
Bleeding Date :		18-10-2007						
Testing Date:		18-10-2007						
Assay :		Mg/Ms		Lot No:				
Positive Cutoff S/P >=		0.5		Dilution :		500		
Mean Titer:		14		Total No. Samples:		62		
G.M.T.		2		Neg/Sus/Pos =		62/0/0		
.		441						
.								
Titer Range Ref. Controls:		R6 (1000-2500)						
Mean Titer Ref. Controls:		R6= 2023						
Sample ID / Well			Raw O.D.	S/P Ratio		Titer	Titer Group/Result	
	-	A01	0.171	0				
	-	B01	0.171	0				
	+	C01	0.659	0				
	+	D01	0.66	0				
01		G01	0.157	0.00001		1	NEG -	
02		H01	0.168	0.00001		1	NEG -	
03		A02	0.157	0.00001		1	NEG -	
04		B02	0.17	0.00001		1	NEG -	
05		C02	0.152	0.00001		1	NEG -	
06		D02	0.159	0.00001		1	NEG -	
07		E02	0.163	0.00001		1	NEG -	
08		F02	0.175	0.008		7	NEG -	
09		G02	0.148	0.00001		1	NEG -	
10		H02	0.151	0.00001		1	NEG -	
11		A03	0.16	0.00001		1	NEG -	
12		B03	0.155	0.00001		1	NEG -	
13		C03	0.153	0.00001		1	NEG -	
14		D03	0.166	0.00001		1	NEG -	
15		E03	0.165	0.00001		1	NEG -	
16		F03	0.172	0.002		2	NEG -	
17		G03	0.178	0.014		13	NEG -	
18		H03	0.163	0.00001		1	NEG -	
19		A04	0.182	0.023		23	NEG -	
20		B04	0.153	0.00001		1	NEG -	
21		C04	0.15	0.00001		1	NEG -	
22		D04	0.152	0.00001		1	NEG -	
23		E04	0.158	0.00001		1	NEG -	

24		F04	0.165	0.00001		1	NEG -	
25		G04	0.152	0.00001		1	NEG -	
26		H04	0.167	0.00001		1	NEG -	
27		A05	0.164	0.00001		1	NEG -	
28		B05	0.154	0.00001		1	NEG -	
29		C05	0.155	0.00001		1	NEG -	
30		D05	0.147	0.00001		1	NEG -	
31		E05	0.176	0.01		9	NEG -	
32		F05	0.162	0.00001		1	NEG -	
33		G05	0.161	0.00001		1	NEG -	
34		H05	0.193	0.045		47	NEG -	
35		A06	0.161	0.00001		1	NEG -	
36		B06	0.154	0.00001		1	NEG -	
37		C06	0.151	0.00001		1	NEG -	
38		D06	0.154	0.00001		1	NEG -	
39		E06	0.161	0.00001		1	NEG -	
40		F06	0.161	0.00001		1	NEG -	
41		G06	0.143	0.00001		1	NEG -	
42		H06	0.164	0.00001		1	NEG -	
43		A07	0.162	0.00001		1	NEG -	
44		B07	0.173	0.004		3	NEG -	
45		C07	0.152	0.00001		1	NEG -	
46		D07	0.157	0.00001		1	NEG -	
47		E07	0.164	0.00001		1	NEG -	
48		F07	0.211	0.082		91	NEG -	
49		G07	0.229	0.119		138	NEG -	
50		H07	0.184	0.027		27	NEG -	
51		A08	0.16	0.00001		1	NEG -	
52		B08	0.167	0.00001		1	NEG -	
53		C08	0.161	0.00001		1	NEG -	
54		D08	0.152	0.00001		1	NEG -	
55		E08	0.157	0.00001		1	NEG -	
56		F08	0.158	0.00001		1	NEG -	
57		G08	0.163	0.00001		1	NEG -	
58		H08	0.171	0.00001		1	NEG -	
59		A09	0.157	0.00001		1	NEG -	
60		B09	0.173	0.004		3	NEG -	
61		C09	0.159	0.00001		1	NEG -	
62		D09	0.346	0.358		463	NEG -	
.								

Table 3 Specificity Panel

Name :	24W+33W						
Bleeding Date :	18-10-2007						
Testing Date:	18-10-2007						
Assay :	Mg/Ms			Lot No:			
Positive Cutoff S/P >=	0.5			Dilution :			
Mean Titer:	6			Total No. Samples:			
G.M.T.	1			Neg/Sus/Pos =			
.	467						
.							
Titer Range Ref. Controls:	R6 (1000-2500)						
Mean Titer Ref. Controls:	R6= 1911						
Sample ID / Well			Raw O.D.	S/P Ratio		Titer	Titer Group/Result
	-	A01	0.164	0			
	-	B01	0.163	0			
	+	C01	0.586	0			
	+	D01	0.624	0			
01		G01	0.151	0.00001		1	NEG -
02		H01	0.161	0.00001		1	NEG -
03		A02	0.147	0.00001		1	NEG -
04		B02	0.147	0.00001		1	NEG -
05		C02	0.141	0.00001		1	NEG -
06		D02	0.146	0.00001		1	NEG -
07		E02	0.145	0.00001		1	NEG -
08		F02	0.149	0.00001		1	NEG -
09		G02	0.148	0.00001		1	NEG -
10		H02	0.166	0.006		5	NEG -
11		A03	0.126	0.00001		1	NEG -
12		B03	0.146	0.00001		1	NEG -
13		C03	0.132	0.00001		1	NEG -
14		D03	0.148	0.00001		1	NEG -
15		E03	0.147	0.00001		1	NEG -
16		F03	0.152	0.00001		1	NEG -
17		G03	0.151	0.00001		1	NEG -
18		H03	0.156	0.00001		1	NEG -
19		A04	0.149	0.00001		1	NEG -
20		B04	0.15	0.00001		1	NEG -
21		C04	0.134	0.00001		1	NEG -
22		D04	0.112	0.00001		1	NEG -
23		E04	0.112	0.00001		1	NEG -
24		F04	0.153	0.00001		1	NEG -

25		G04	0.156	0.00001		1	NEG -
26		H04	0.161	0.00001		1	NEG -
27		A05	0.134	0.00001		1	NEG -
28		B05	0.148	0.00001		1	NEG -
29		C05	0.144	0.00001		1	NEG -
30		D05	0.131	0.00001		1	NEG -
31		E05	0.148	0.00001		1	NEG -
32		F05	0.133	0.00001		1	NEG -
33		G05	0.131	0.00001		1	NEG -
34		H05	0.167	0.008		7	NEG -
35		A06	0.127	0.00001		1	NEG -
36		B06	0.146	0.00001		1	NEG -
37		C06	0.1	0.00001		1	NEG -
38		D06	0.152	0.00001		1	NEG -
39		E06	0.152	0.00001		1	NEG -
40		F06	0.174	0.024		24	NEG -
41		G06	0.169	0.012		11	NEG -
42		H06	0.146	0.00001		1	NEG -
43		A07	0.232	0.155		184	NEG -
.							

BioChek (c)

DATA SHEETS

MONOSPECIFIC SAMPLE PANEL
Monospecific samples containing antibodies to various Mycoplasmas.

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

Procedure
A sample panel monospecific for antibodies of Mycoplasma pathogens common in poultry was tested on the BioChek MgMS test.

Results / Conclusion
The results are shown in Table 4

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

Table 4 Monospecific Panel

	Biochek MgMsHS	
Antiserum	**S/P**	**result**
M. synoviae	**3.1**	**pos**
M. gallisepticum	**4.8**	**pos**
M. galinaceum	0.0	neg
M. gallinar	0.0	neg
M. iowae	0.0	neg
M. meleagridis	0.0	neg
M. iners	0.0	neg
M. pullorum	0.0	neg
M. gallopavonis	0.0	neg
M. imitans	0.1	neg

DATA SHEETS

MONOSPECIFIC SAMPLE PANEL 2
Monospecific samples containing antibodies to various viruses.

Purpose
To determine if the BioChek MgMS 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 MgMS test.

Results / Conclusion
The results are shown in Table 5

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

Table 5Monospecific Panel

Sample ID	S/P Ratio	RESULT			
4/91DEV	0.039	NEG	D274INT	0.018	NEG
4/91INT	0.018	NEG	D3128	0.011	NEG
793BVLA	0.018	NEG	D8880	0.022	NEG
adeno	0.018	NEG	ECOLI1	0.081	NEG
AE	0.018	NEG	ECOLI2	0.018	NEG
CR88	0.018	NEG	Fpox	0.018	NEG
CR98	0.018	NEG	IBD	0.017	NEG
D1466	0.018	NEG	ILT	0.018	NEG
D1466INT	0.018	NEG	ILTAGP	0.018	NEG
D274	0.011	NEG	M41	0.028	NEG
M41INT	0.018	NEG	REO1133	0.018	NEG
Mg	**2**	**POS**	REO2534	0.018	NEG
Ms	**1.3**	**POS**	TRTA	0.018	NEG
PMV1	0.018	NEG	TRTC	0.013	NEG
PMV3	0.018	NEG			

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

DATA SHEETS

RING TRIAL

Here is the summary of the Deventer ring Trial 2006. 8 samples were tested:

Sample number	Origin (all sera are pooled sera)
#1	Nobilis Mg S6/85 strain in 4-week-old SPF layers, sample taken at 21 d.p.i*. (normally tests negative on ELISA
#2	Ms ATCC 25204 in 26-week-old SPF layers, sample taken at 35 d.p.i.
#3	Nobilis Mg S6/85 + Ms ATCC 25204 in SPF layers, chronic phase
#4	SPF serum (1 year old SPF layers)
#5	Ms ATCC 25204 + Ms Associated Amyloidosis strain 30-week-old SPF layers, sample taken at 35 d.p.i.
#6	Ms Associated Amyloidosis strain 4-week-old SPF layers, sample taken at 14 d.p.i.
#7	Mg field strain in 4-week-old SPF layers, sample taken at 28 d.p.i.
#8	Poulvac Mg (inactivated vaccine) in 4-week-old SPF layers, sample taken at 14 d.p.v**.
* d.p.i. = days post inoculation ** d.p.v. = days post vaccination	

Mg Ringtrial AHS Deventer 2006
suspects results have been counted as negative
in the table the result 0/2/21 means 0 positives/2 suspect/21 negatives

Table 1

sample	1	2	3	4	5	6	7	8
status	neg	Ms	Mg/Ms	neg	Ms	Ms	Mg	Mg
BC Mg	0/2/21	0/2/21	23/0/0	1/1/21	0/2/21	0/0/23	23/0/0	23/0/0
Intervet RPA Mg	26/3/6	3/0/32	34/1/0	2/0/33	5/0/29	4/0/31	32/1/2	31/1/3

BioChek Mg specificity **99.13%**
BioChek Mg sensitivity **100%**

Intervet RPA specificity **98.29%**
Intervet RPA sensitivity **94.29%**

Ms Ringtrial AHS Deventer 2006
suspects results have been counted as negative
in the table the result 0/2/21 means 0 positives/2 suspect/21 negatives

table 2

sample	1	2	3	4	5	6	7	8
status	neg	Ms	Mg/Ms	neg	Ms	Ms	Mg	Mg
BC Ms	0/0/14	13/0/1	13/0/1	0/1/13	13/0/1	1/0/13	0/1/13	0/1/13
BC Ms HS	0/0/2	2/0/0	2/0/0	0/0/2	2/0/0	2/0/0	0/0/2	0/0/2
Intervet RPA Ms	1/0/28	25/3/1	26/1/2	1/0/28	29/0/0	27/0/2	0/0/29	4/2/23

BioChek Ms specificity **100%**
BioChek Ms sensitivity **71%**

BioChek Ms HS specificity **100%**
BioChek Ms HS sensitivity **100%**

Intervet Ms RPA specificity **94.83%**
Intervet Ms RPA sensitivity **92.24%**

MgMs Ringtrial AHS Deventer 2006

suspects results have been counted as negative
in the table the result 0/2/21 means 0 positives/2 suspect/21 negatives

table 3

sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
status	neg	Ms	Mg/Ms	neg	Ms	Ms	Mg	Mg
BC MgMs	0/0/4	4/0/0	4/0/0	0/0/4	4/0/0	0/0/4	4/0/0	4/0/0
	+	+	+	-	+	+	+	+
BioChek MgMs specificity			**100%**					
BioChek MgMs sensitivity			**75%**					