BioChek
SMART VETERINARY DIAGNOSTICS

MG

Data Pack

Mycoplasma gallisepticum Antibody Test Kit
(Detects antibodies to mycoplasma gallisepticum 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

Specificity

Highly Specific (>98%) to antibodies against Mycoplasma gallisepticum.

Sensitivity

Sensitivity similar to Rapid Plate Agglutination (RPA). Will detect positive samples 14 days post infection.

Reproducibility

Intra-Plate CV's lower than 10%.
Batch to Batch variability lower than 10%

Applications

Screening

The most common use of the BioChek Mg ELISA is screening flocks for positives. When positive samples are found confirm with alternative methods such as culture or PCR.

The Mg 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 gallisepticum Antibody Test Kit

Catalogue Code CK 114

Description of Test

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

Reagents provided:

1. **Mg 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 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 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 positive control has been carefully standardised to represent significant amounts of antibody to Mg 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 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 = 1.1 (log10 S/P) + 3.156

Antilog = Titre

S/P value	Titre Range	Antibody status
0.499 or less	667 or less	Negative
0.500 or greater	668 or greater	Positive

For confirmation of status additional alternative testing should be performed.

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KI/CK114REV04

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 MG ELISA.

Procedure
36 samples from 12 week old SPF white Leghorns were obtained (Lohmann Cuxhaven Germany) and assayed using the standard protocol for the BioChek MG ELISA.

Results/Conclusion
The results are shown in the Table 1

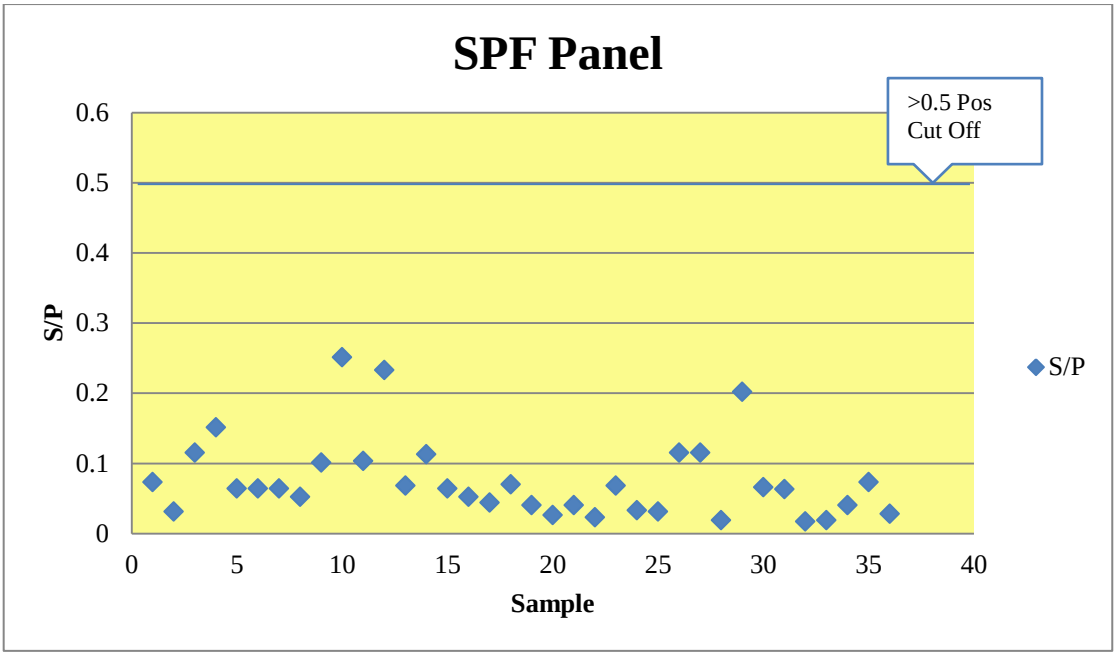
In graph I, the S/P value of each sample is plotted on the Y-axis, the sample number on the X-axis. The mean S/P value was .0327 and the Standard deviation was .0229. The S/P values were well below the positive cut-off value of 0.20

The data demonstrate that the BioChek MG ELISA has a100% specificity on this sample panel.

Table I Specificity, Negative population

ELISA MG SPF Negative Panel			Mean S/P	0.0759		Kit Lot No.	FS4310
			St dev	0.0572			
Sample							
Number	SP	Result					
1	0.073	NEG					
2	0.031	NEG					
3	0.115	NEG					
4	0.151	NEG					
5	0.064	NEG					
6	0.064	NEG					
7	0.064	NEG					
8	0.052	NEG					
9	0.101	NEG					
10	0.251	NEG					
11	0.103	NEG					
12	0.233	NEG					
13	0.068	NEG					
14	0.113	NEG					
15	0.064	NEG					
16	0.052	NEG					
17	0.044	NEG					
18	0.070	NEG					
19	0.040	NEG					
20	0.026	NEG					
21	0.040	NEG					
22	0.023	NEG					
23	0.068	NEG					
24	0.033	NEG					
25	0.031	NEG					
26	0.115	NEG					
27	0.115	NEG					
28	0.019	NEG					
29	0.202	NEG					
30	0.066	NEG					
31	0.063	NEG					
32	0.017	NEG					
33	0.019	NEG					
34	0.040	NEG					
35	0.073	NEG					
36	0.028	NEG					

Graph 1 Specificity, Negative Population



DATA SHEETS

MONO-SPECIFIC SERUM PANEL
Serum samples positive for MG antibody, negative for other avian pathogens.

Purpose
To determine if the BioChek MG 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 MG test . The following monospecific sera were tested on the BioChek MG test: Avian Paramyxovirus serotype 1 (Newcastle Disease Virus), Avian Paramyxovirus serotype 3 , Avian Pneumovirus Type A and C, Infectious Laringotracheitis, Infectious Bronchitis serotypes 4/91, cr88, cr98,M41, D274, D1466, D3128, D8880 , Egg Drop Syndrome (Adenovirus), Fowl Pox, Reo virus S1133 & S2534S, Mycoplasma Gallisepticum, Mycoplasma Synoviae, Infectious Bursal Disease, and Avian Encephalomyelitis.
Also a Mycoplasma spp. Panel, mono-specific for antibodies for M. gallopavonis, M. iners, M. iowa, M. meleagridis, M. pullorum, M. gallinaceum, M. gallinarum, M. synovea, and M. gallisepticum was tested on the BioChek MG test to determine cross-reactivity with other avian Mycoplasma pathogens.

Results/Conclusion
Results are presented in Table II and IIa.

The BioChek MG ELISA tested negative on all samples tested, with exception of the MG positive samples in both sample panels.

Only the mono-specific serum samples for MG tested positive on the BioChek MG ELISA. Also within the Mycoplasma group of avian pathogens , MG ELISA does not react with other serum samples, other then MG.

The conclusion is that the BioChek MG ELISA is specific to antibodies, belonging to M. gallisepticum.

Table II. Specificity, Mono-Specific serum panel

Interpretation of results BioChek Mg ELISA
S/P <.5 Negative
S/P =>.5 Positive

Name : BC monospecific sample panel
Code :
Bleeding Date : 26/02/2002
Assay : Mg Lot: FS3726

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	0.068	NEG	TRTA	0.018	NEG
PMV1	0.018	NEG	TRTC	0.013	NEG
PMV3	0.018	NEG			

Table IIa. Specificity, Mono-Specific Mycoplasma spp. serum panel

Interpretation of results BioChek Mg ELISA
S/P <.5 Negative
S/P =>.5 Positive

Name :	Mycoplasma specificity panel
Company :	SPAFAS monospecific samples
Code :	SPECIFICITY
BleedDate :	28-07-1999
Assay :	Mg
Dilution:	1 : 500

Sample		Raw O.D.	S/P Ratio	RESULT
-	A01	0.124		
-	A02	0.124		
+	A03	0.665		
+	A04	0.665		
M. gallopavonis	B09	0.122	0.018	NEG
M. gallopavonis	B10	0.122	0.018	NEG
M. iners	B05	0.119	0.018	NEG
M. iners	B06	0.125	0.002	NEG
M. iowa	B01	0.129	0.009	NEG
M.iowa	B02	0.127	0.006	NEG
M. meleagridis	B03	0.137	0.024	NEG
M. meleagridis	B04	0.134	0.018	NEG
M. pullorum	B07	0.136	0.022	NEG
M. pullorum	B08	0.128	0.007	NEG
M. gallinaceum	A09	0.119	0.018	NEG
M. gallinaceum	A10	0.117	0.018	NEG
M. gallinarum	A11	0.128	0.007	NEG
M. gallinarum	A12	0.126	0.004	NEG
M. synovea	A07	0.178	0.1	NEG
M. synovea	A08	0.178	0.1	NEG
M. gallisepticum	**A05**	**2.984**	**5.287**	**POS**
M. gallisepticum	**A06**	**3.102**	**5.505**	**POS**

DATA SHEETS

SENSITIVITY

Purpose of the trial: Date: 27 september 2005

Trial on sensitivity and specificity of the BioChek Mg ELISA test
Description of the trial:
6 SPF chickens were infected with Mg at 3 weeks of age.
6 other SPF chicks were infected with Ms at 3 weeks of age.
Serum samples were collected every week.
At the end of the trial the samples were sent to BioChek UK Ltd.

Results
Sensitivity: Both RPA and the BC ELISA test positive 14 days post challenge
HI tests positive 21 days post challenge
Both RPA and the BioChek ELISA test negative on Ms challenged chickens

Mg temporal samples

		% positive		% positive	
age	**Days P.I**	**RPA Mg**	**HI Mg**	**BC Mg std**	**BC Mg new**
3W	D00	0	0	0	0
4W	D07	0	0	0	0
5W	D14	83	0	33	33
6W	D21	100	81	60	50

Ms temporal samples

		% positive		% positive
age	**Days P.I**	**RPA Mg**	**HI Ms**	**BioChek Mg**
3W	D00	0	0	0
4W	D07	0	0	0
5W	D14	0	50	0
6W	D21	0	100	0

Samples provided by Dr. S.H. Kleven PDRC Athens, GA in february 2005
RPA and HI performed by PDRC Athens Greorgia
BC (= BioChek) Mg performed by BioChek UK Ltd.

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 MG was tested on several batches of the BioChek MG ELISA. The purpose of the trail is to assess batch to batch reproducibility.

Procedure

A known, pre-diluted MG sample, RF06 is assayed in duplicate on 7 different production batches of MG kits. Mean S/P values, standard deviation, and C.V. are calculated to assess 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 follows:
For the RF06 sample results were:

Mean S/P	0.93
SD	0.08
%CV	8.71

The data demonstrates that there is limited variation (< 10%) when comparing results from various production batches of the BioChek MG ELISA

IV Reproducibility of the BioChek MG ELISA **Date** **01-03-2006**

Sample High: prediluted serum RF06 containing positive titers of antibodies against MG
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 HIGH**
RF06	FS4248	01-07-2005	MG	0.113	0.114	0.699	0.722	0.592	0.600	0.8100	2	**Mean S/P** **0.93**
RF06	FS4262	01-08-2005	MG	0.172	0.170	0.896	0.934	0.900	0.868	0.9600	2	**SD** **0.08**
RF06	FS4278	14-09-2005	MG	0.130	0.131	0.729	0.736	0.662	0.682	0.9000	2	**%CV** **8.71**
RF06	FS4292	14-10-2005	MG	0.149	0.148	1.013	0.967	0.849	0.901	0.8600	2	
RF06	FS4310	18-11-2005	MG	0.148	0.146	0.616	0.618	0.607	0.574	0.9500	2	
RF06	FS4324	09-12-2005	MG	0.171	0.173	0.899	0.885	0.905	0.858	0.9900	2	
RF06	FS4352	01-03-2006	MG	0.138	0.139	0.724	0.726	0.765	0.736	1.0500	2	

Trial 2: Intra-Assay Reproducibility

Purpose

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

Procedure

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

Results/Conclusion:

Results are shown in the table V.

The %CV of the sample (RF06) is 5.97 %.

Table V. Intra-plate Reproducibility of BioChek MG ELISA

BioChek Intraplate reproducibility study

Assay	**MG**	**Lot**	**FS4202**
date	**13-12-2005**		
Mean OD	**0.597**		
st dev	**0.04**		
%CV	**5.97**		

MG

	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	0.546	0.502	0.579	0.617	0.615	0.675	0.559	0.66	0.606	0.585	0.667	0.651
Row B	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06
OD	0.567	0.559	0.593	0.612	0.605	0.601	0.606	0.619	0.625	0.587	0.574	0.626
Row C	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	**NEG**
OD	0.558	0.534	0.565	0.579	0.565	0.586	0.583	0.585	0.606	0.581	0.574	**0.137**
Row D	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	**NEG**
OD	0.534	0.511	0.551	0.578	0.529	0.594	0.597	0.588	0.603	0.595	0.588	**0.139**
Row E	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	**POS**
OD	0.555	0.57	0.573	0.579	0.614	0.597	0.602	0.583	0.615	0.611	0.626	**0.612**
Row F	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	**POS**
OD	0.535	0.536	0.569	0.557	0.583	0.56	0.595	0.596	0.614	0.614	0.625	**0.602**
Row G	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	
OD	0.581	0.588	0.62	0.59	0.619	0.655	0.638	0.636	0.645	0.639	0.642	
Row H	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	RF06	
OD	0.637	0.607	0.602	0.61	0.636	0.648	0.634	0.628	0.656	0.625	0.647	