BioChek
SMART VETERINARY DIAGNOSTICS

EDS

Data Pack

Egg Drop Syndrome Antibody ELISA
(Detects antibodies to EDS)

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

Positive reactions 10 - 21 days after infection

Specificity

Specific for EDS 76 virus, doesn't cross react with other ADENO viruses.

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.

Field infection

About 10 - 21 days after infection seroconversion will show.

BioChek Poultry Immunoassays
Egg Drop Syndrome Antibody Test Kit

Catalogue Code CK 112

Description of Test

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

SAMPLE
INSERT

Reagents provided:

1. **EDS 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 EDS 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!!

SAMPLE INSERT

Test procedure:

1. Remove EDS 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 EDS positive control has been carefully standardised to represent significant amounts of antibody to EDS 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-EDS antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

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

SAMPLE INSERT

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 = 1.14 * (log10 S/P) + 3.156

Antilog = Titre

S/P value	**Titre Range**	**Antibody status**
0.499 or less	649 or less	Negative
0.500 or greater	650 or greater	Positive

For confirmation of status additional alternative testing should be performed.

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Manufacturer:
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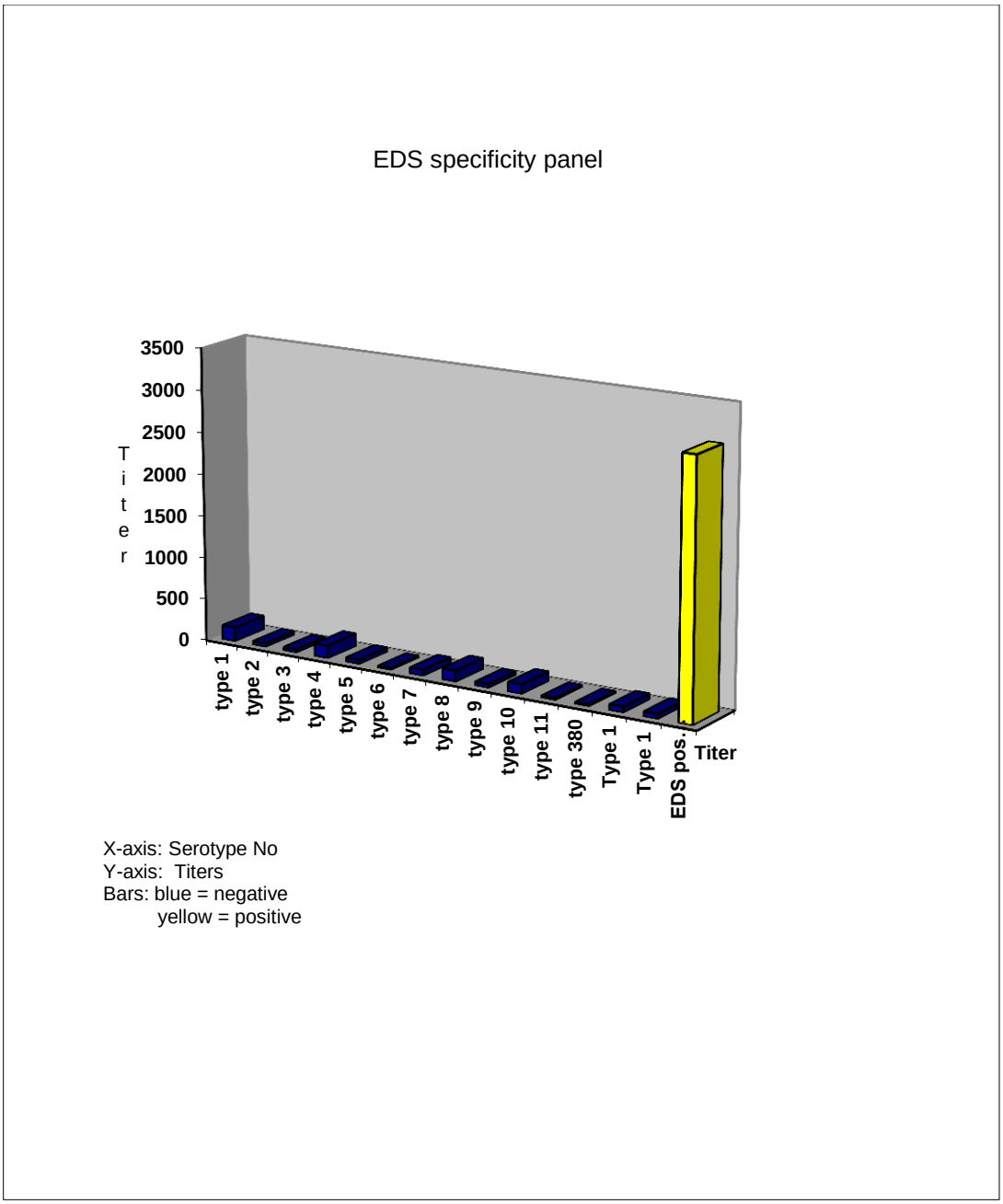
KI/CK112REV04

DATA SHEETS

SEROTYPES

Samples monospecific for various known serotypes were tested on the BioChek EDS ELISA. All serotypes other than EDS test negative.



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 EDS ELISA.

Procedure

30 samples from 6 different flocks of 33 week old SPF Broiler Breeders were obtained and assayed using the standard protocol for the BioChek EDS ELISA

Results / Conclusion

The results are shown in Table 1.

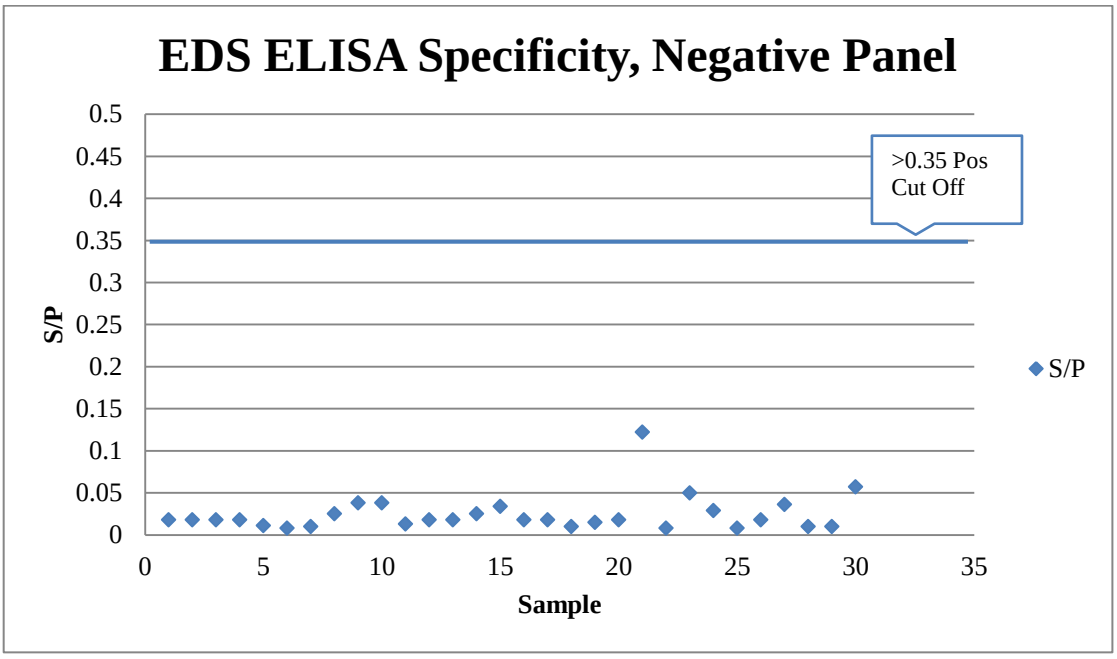
The results have been plotted on Graph1 showing S/P value against sample number.

The positive cut off for the BioChek EDS ELISA is an S/P value of 0.35. The data demonstrates that the BioChek EDS ELISA has 100% specificity on this sample panel.

Table 1 EDS Specificity, Negative Panel

	Sample ID	S/P Ratio	Titer	
A	01	0.018	15	NEG -
	02	0.018	15	NEG -
	03	0.018	15	NEG -
	04	0.018	15	NEG -
	05	0.011	8	NEG -
B	01	0.008	6	NEG -
	02	0.01	8	NEG -
	03	0.025	21	NEG -
	04	0.038	34	NEG -
	05	0.038	34	NEG -
C	01	0.013	10	NEG -
	02	0.018	15	NEG -
	03	0.018	15	NEG -
	04	0.025	21	NEG -
	05	0.034	30	NEG -
D	01	0.018	15	NEG -
	02	0.018	15	NEG -
	03	0.01	8	NEG -
	04	0.015	12	NEG -
	05	0.018	15	NEG -
E	01	0.122	130	NEG -
	02	0.008	6	NEG -
	03	0.05	47	NEG -
	04	0.029	25	NEG -
	05	0.008	6	NEG -
F	01	0.018	15	NEG -
	02	0.036	32	NEG -
	03	0.01	8	NEG -
	04	0.01	8	NEG -
	05	0.057	55	NEG -

Graph1 EDS Specificity, Negative Panel



DATA SHEETS

MONOSPECIFIC SAMPLE PANEL
Monospecific samples containing antibodies to various viruses.

Purpose
To determine if the BioChek EDS 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 EDS test.

Results / Conclusion
The results are shown in Table 2

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

Table 2 Monospecific Sample Panel

Name :	MONOSPECIFICS sample panel		
Bleeding Date :	25/02/2002		
Assay :	BioChek EDS	Lot No:	FS3712
		Dilution :	'1:500

Interpretation results			
S/P value	**Titre Range**		**Antibody status**
.349 or less	432 or less		Negative
.350 - 0.499	433 - 649		Suspect
.500 or greater	650 or greater		Positive

Sample ID	S/P Ratio	Sample ID	S/P Ratio
4/91DEV	0.019	ECOLI2	0.323
4/91INT	0.014	Fpox	0.012
793BVLA	0.008	IBD	0.019
adeno	0.005	ILT	0.017
AE	0.002	ILTAGP	0.008
CR88	0.002	M41	0.008
CR98	0.02	M41INT	0.014
D1466	0.032	Mg	0.017
D1466INT	0.007	Ms	0.02
D274	0.019	PMV1	0.017
D274INT	0.034	PMV3	0.036
D3128	0.005	REO1133	0.005
D8880	0.034	REO2534	0.008
ECOLI1	0.301	TRTA	0.017
EDS	**1.941**	TRTC	0.017

DATA SHEETS

SENSITIVITY

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

Procedure
A flock were experimentally infected by EDS 76 virus. Samples were then taken serially for up to 50 days post infection.

Results/Conclusion
The results are shown below in Table 3 and Graph 2

The results below demonstrate that the BioChek EDS ELISA detects antibodies from 10 days post vaccination.

Table 3 Samples from 0 days to 50 days post vaccination

BioChek EDS ELISA

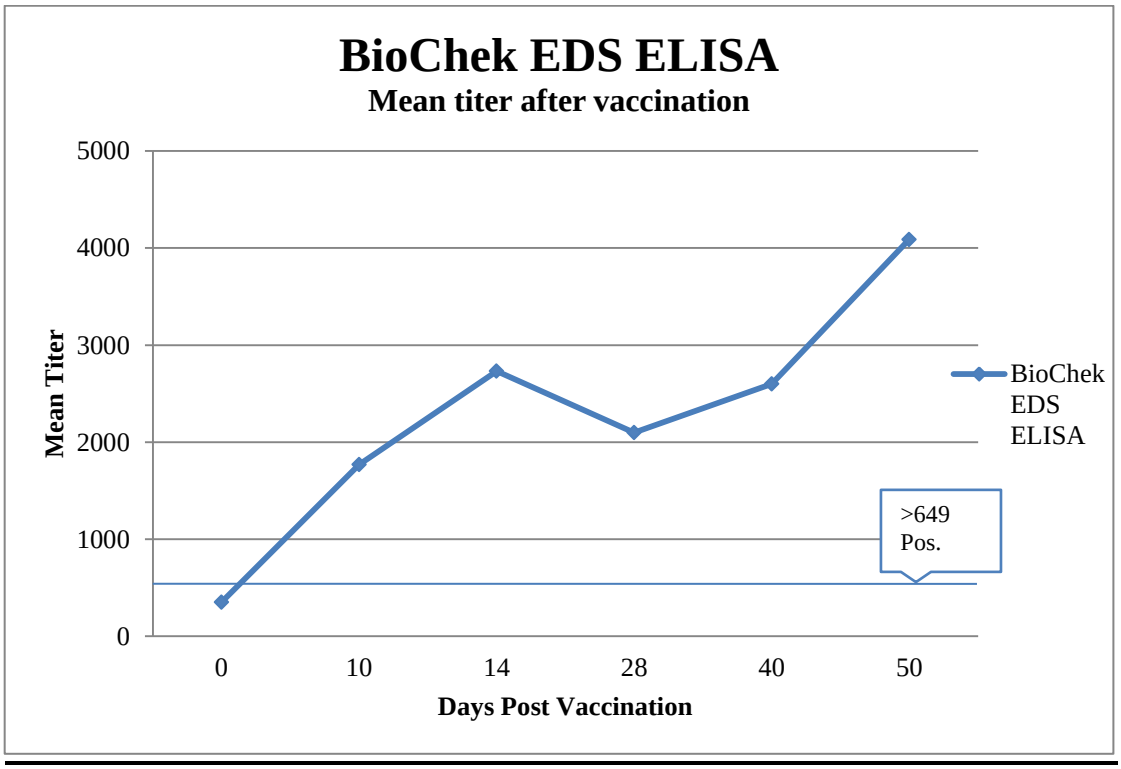
Flock experimentally infected by EDS 76 virus.
Samples collected serially up to 50 days post vaccination

Flock:	field samples		
Code :	00093		
Bleeding Date :	04/10/2001		
Assay :	EDS	Lot No:	FS 3656
Mean Titer:	2381	Dilution :	500
G.M.T.:	1663	%CV :	73.22

Sample ID	S/P Ratio	BC Titer	Days
01	0.198	226	0
02	0.256	303	0
03	0.414	524	0
04	1.716	**2651**	10
05	0.631	**847**	10
06	1.229	**1812**	10
07	0.687	**934**	14
08	1.848	**2884**	14
09	2.665	**4378**	14
10	0.511	**666**	28
11	0.819	**1141**	28
12	2.727	**4494**	28
13	3.305	**5596**	40
14	0.561	**741**	40
15	1.475	**2231**	40
16	1.241	**1832**	40
17	3.195	**5384**	50
18	2.34	**3775**	50
19	2.314	**3727**	50
20	2.172	**3468**	50

* Results in bold are positive

Graph 2Mean titer after vaccination



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 EDS MEDIUM containing antibodies to EDS was tested on several batches of the BioChek EDS ELISA.
The purpose of the trial is to assess batch to batch reproducibility.

Procedure

A known, pre-diluted EDS sample, (EDS MEDIUM) is assayed in duplicate on 6 different production batches of EDS 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 5 batches is as following:

For the EDS MEDIUM sample results were:

Mean Titre	2010
SD	137
%CV	7

Table IV reproducibility:

Assay: EDS

Mean Titer For All Flocks	2010	
Standard Deviation of Mean Flock Titers:	137	
Coefficient of Variation of Mean Flock Titers:	7	%

Name	Company	MEAN
MEDIUM	FS4641	2065
MEDIUM	FS4665	2060
MEDIUM	FS4693	2041
MEDIUM	FS4718	2167
MEDIUM	FS4760	2021
MEDIUM	FS4793	2012
MEDIUM	FS4641	2060
MEDIUM	FS4641	2048
MEDIUM	FS4641	1948
MEDIUM	FS4665	2038
MEDIUM	FS4665	2158
MEDIUM	FS4665	2066
MEDIUM	FS4693	1874
MEDIUM	FS4693	1722
MEDIUM	FS4693	1821
MEDIUM	FS4693	1992
MEDIUM	FS4718	2332
MEDIUM	FS4718	2164
MEDIUM	FS4718	2156
MEDIUM	FS4760	1835
MEDIUM	FS4760	1919
MEDIUM	FS4760	1854
MEDIUM	FS4793	1850
MEDIUM	FS4793	1960
MEDIUM	FS4793	2085

These data demonstrate that there is limited variation (< 15%) , when comparing results from various production batches of the BioChek EDS ELISA

Trial 2: Intra-Assay Reproducibility

Purpose

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

Procedure

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

Results/Conclusion

The %CV of the sample (EDS HIGH) is 2.98 %.

DATA SHEETS

COMPARISON WITH HI

BioChek EDS ELISA

Flock infected by EDS 76 virus. Comparison of HI with BioChek EDS ELISA.
Samples, HI and BioChek ELISA by Ghen, Japan

Results:

HI	**20 x pos**	**sensitivity**	**100%**
ELISA	**17 x pos**	**sensitivity**	**85%**

Interpretation BioChek ELISA

S/P value	**Titre Range**		**Antibody status**	
.349 or less	432 or less		Negative	
.350 - 0.499	433 - 649		Suspect	
.500 or greater	650 or greater		Positive	

Interpretation HI	
HI < 4	=NEG

Flock: field samples
Code : 00093
Bleeding Date : 04/10/2001

Assay :	EDS	Lot No:	FS 3656
Mean Titer:	2381	Dilution :	500
G.M.T.:	1663	%CV :	73.22

Sample ID	S/P Ratio	HI	BC Titer
01	0.198	**32***	226
02	0.256	**32**	303
03	0.414	**32**	524
04	1.716	**64**	**2651**
05	0.631	**64**	**847**
06	1.229	**128**	**1812**
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15	1.475	**32**	**2231**
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19	2.314	**256**	**3727**
20	2.172	**256**	**3468**

* Results in bold are positive