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Header 1

List View

General Information | Contact | Default Values | Discount | Document Information | Clarification Request

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**State of West Virginia
 Solicitation Response**

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Solicitation Description: AVIAN INFLUENZA VIRUS TEST KITS/ ELISA
Proc Type: Central Master Agreement

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FOR INFORMATION CONTACT THE BUYER

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Vendor Signature X **FEIN#** **DATE**

All offers subject to all terms and conditions contained in this solicitation

Line	Comm Ln Desc	Qty	Unit Issue	Unit Price	Ln Total Or Contract Amount
1	Avian Influenza Virus Test Kits / ELISA	180.00000	EA	376.000000	67680.00

Comm Code	Manufacturer	Specification	Model #
41116126			

Commodity Line Comments: Shipping included at the USDA mandatory FED EX overnight service rate. The BioChek AI ELISA kit is USDA licensed and the ONLY AI ELISA kit to have OIE certification for international use and testing. The highest certification available for diagnostic test kits. If the lab receives samples from chickens being shipped internationally this is a great advantage. BioChek is the only supplier to provide an external control to validate the quality of results being reported.

Extended Description:

Unit Price must include all shipping and handling charges



AI

Data Pack

Avian Influenza Antibody ELISA
(Detects antibodies to Type A avian influenza virus)

CONTENTS

Summary	Page 3
The Kit	
Key Performance Features	
Applications	
Package Insert	Pages 4-6
Description of test	
Reagents provided	
Materials and equipment required	
Warnings and precautions	
Reagent preparation	
Sample preparation	
Test procedure	
Results	
Interpretation of results	
Data Sheets	Pages 7-11
Specificity: Negative population	
Monospecific sample panel	
Sensitivity	
Comparison with HI	
Avian Influenza: An Overview	Pages 12 -14

SUMMARY

Kit

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

Key Performance Features

General:

- Detect antibodies against type A avian influenza in Chicken and Turkey sera
- Avian Influenza is caused by Influenza type A; it belongs to the group of Orthomyxoviridea. The Influenza virus is subtyped based on surface antigens H (Haemagglutinin) and N (Neuraminidase), there are 15 types of H, H1 – H15 and 9 types of N (N1-N9).

The following AI serotypes from type A have been tested on the BioChek AI ELISA. Samples originate VLA U.K. All samples tested positive:

H1N2	H8N4	H15N6	H2N3	H9N2	
H3N2	H10N9	H4N6	H11N6	H5N1	H12N5
H6N8	H13N6	H7N7	H14N6		

Sensitivity

Comparison studies from various field cases suggest that the BC AI was at least as sensitive as HI. In some field cases the results show that the BC ELISA is more sensitive than HI. This was the case in broilers of 36 days old confirmed positive for H9 by HI. 35 out of 35 samples or 100% tested positive on the BC AI, 11 out 35 samples or 31% tested positive on HI (VLA-Weybridge, UK).

Specificity

>98% on field flocks

Applications

Field infection

About 10 - 20 days after infection seroconversion will show. Positive results means that the flock has been in contact with Avian Influenza virus.

When positive alternative methods such as HI can be used to determine the serotype.

Vaccination check

Test flock after AI vaccination in order to establish efficiency of vaccination. Answers to key questions like “did the vaccine actually stimulate the immune system”, will be answered.

Test 2- 5 weeks after live vaccination and 5 - 10 weeks after vaccination with inactivated vaccine.

BioChek Poultry Immunoassays

Avian Influenza Antibody Test Kit

Catalogue Code CK 121

Description of Test

The AI ELISA kit will measure the amount of antibody to AI in the serum of chickens and Turkeys. Microtitre plates have been pre-coated with inactivated AI antigen. Serum samples are diluted and added to the microtitre wells where any anti-AI 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-AI antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow color is developed if anti-AI antibody is present and the intensity is directly related to the amount of anti-AI present in the sample.

Reagents provided

1. **AI Coated plates.** Inactivated viral antigen on microtitre plates
2. **Conjugate reagent.** Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilizers, 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 stabilizers 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 stabilizers and sodium azide preservative (0.1% w/v)
9. **Positive Control.** Antibodies specific to AI in Phosphate Buffer with protein stabilizers 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 AI 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 (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 **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 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 0.3.

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 AI positive control has been carefully standardized to represent significant amounts of antibody to AI 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 .5 or greater contain anti-AI 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

$$\text{Log}_{10} \text{Titre} = 1.1 * \text{Log}(\text{SP}) + 3.156$$

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

S/P value	Titre Range	Antibody status
.499 or less	667 or less	Negative
.500 or greater	668 or greater	Positive

Additional alternative testing should be performed on any suspect or positive samples in order to obtain a confirmed positive diagnosis of Avian Influenza within a chicken or Turkey flock.

BioChek has available a software program which can be used with the AI 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 AI ELISA.

Procedure

102 samples from SPF Broiler Breeders were obtained (Deveter, Holland) and assayed using the standard protocol for the BioChek AI ELISA

Results/Conclusion

The results are shown in the following tables.

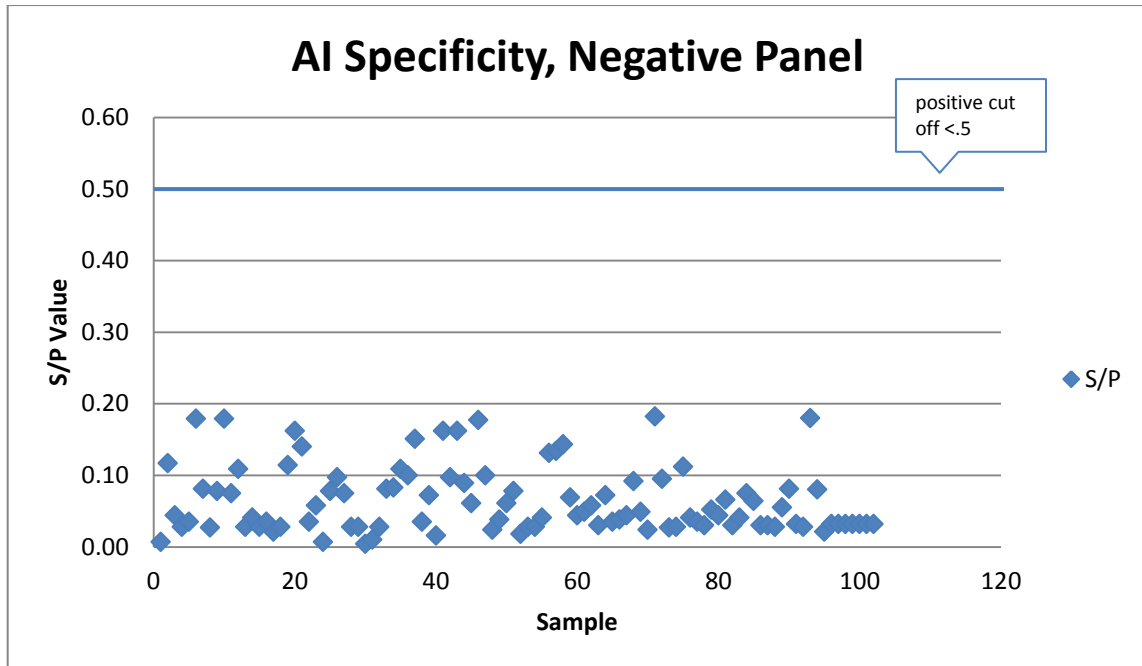
The results have been plotted on Graph 1 showing S/P value against sample number.

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

Table 1 Specificity, Negative Panel

Field samples. Breeders presumably negative for AI															
All samples test negative. On this sample panel the BioChek AI ELISA is 100% specific.															
Positive cutoff S/P > .5															
sample	S/P	titer	result	sample	S/P	titer	result	sample	S/P	titer	result	sample	S/P	titer	result
01	0.01	10	NEG -	26	0.10	176	NEG -	51	0.08	139	NEG -	76	0.04	68	NEG -
02	0.12	217	NEG -	27	0.08	133	NEG -	52	0.02	28	NEG -	77	0.04	57	NEG -
03	0.04	74	NEG -	28	0.03	45	NEG -	53	0.03	45	NEG -	78	0.03	49	NEG -
04	0.03	45	NEG -	29	0.03	45	NEG -	54	0.03	45	NEG -	79	0.05	89	NEG -
05	0.04	57	NEG -	30	0.00	5	NEG -	55	0.04	68	NEG -	80	0.04	74	NEG -
06	0.18	346	NEG -	31	0.01	14	NEG -	56	0.13	245	NEG -	81	0.07	115	NEG -
07	0.08	145	NEG -	32	0.03	45	NEG -	57	0.13	252	NEG -	82	0.03	49	NEG -
08	0.03	43	NEG -	33	0.08	145	NEG -	58	0.14	270	NEG -	83	0.04	68	NEG -
09	0.08	139	NEG -	34	0.08	149	NEG -	59	0.07	121	NEG -	84	0.08	133	NEG -
10	0.18	346	NEG -	35	0.11	201	NEG -	60	0.04	74	NEG -	85	0.06	112	NEG -
11	0.08	133	NEG -	36	0.10	182	NEG -	61	0.05	83	NEG -	86	0.03	49	NEG -
12	0.11	201	NEG -	37	0.15	287	NEG -	62	0.06	100	NEG -	87	0.03	49	NEG -
13	0.03	45	NEG -	38	0.04	57	NEG -	63	0.03	49	NEG -	88	0.03	45	NEG -
14	0.04	68	NEG -	39	0.07	127	NEG -	64	0.07	127	NEG -	89	0.06	94	NEG -
15	0.03	45	NEG -	40	0.02	24	NEG -	65	0.04	57	NEG -	90	0.08	145	NEG -
16	0.04	57	NEG -	41	0.16	310	NEG -	66	0.04	63	NEG -	91	0.03	52	NEG -
17	0.02	33	NEG -	42	0.10	176	NEG -	67	0.04	74	NEG -	92	0.03	45	NEG -
18	0.03	45	NEG -	43	0.16	310	NEG -	68	0.09	166	NEG -	93	0.18	348	NEG -
19	0.11	211	NEG -	44	0.09	160	NEG -	69	0.05	83	NEG -	94	0.08	143	NEG -
20	0.16	310	NEG -	45	0.06	106	NEG -	70	0.02	38	NEG -	95	0.02	33	NEG -
21	0.14	264	NEG -	46	0.18	342	NEG -	71	0.18	352	NEG -	96	0.03	52	NEG -
22	0.04	57	NEG -	47	0.10	182	NEG -	72	0.10	172	NEG -	97	0.03	52	NEG -
23	0.06	100	NEG -	48	0.02	38	NEG -	73	0.03	43	NEG -	98	0.03	52	NEG -
24	0.01	10	NEG -	49	0.04	63	NEG -	74	0.03	45	NEG -	99	0.03	52	NEG -
25	0.08	139	NEG -	50	0.06	106	NEG -	75	0.11	207	NEG -	100	0.03	52	NEG -
												101	0.03	52	NEG -
												102	0.03	52	NEG -

Graph 1 AI Specificity, Negative Panel



DATA SHEETS

MONOSPECIFIC PANEL

Monospecific samples containing antibodies to various viruses.

Purpose

To determine if the BioChek AI 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 AI ELISA.

Results / Conclusion

The results are shown in Table 2

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

Table 2 AI Monospecifics Panel

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

Interpretation results		
S/P value	Titre Range	Antibody status
.349 or less	432 or less	Negative
.500 or greater	650 or greater	Positive

Sample ID	S/P Ratio
4/91DEV	0.13
4/91INT	0.05
793BVLA	0.03
adeno	0.03
AE	0.03
CR88	0.01
CR98	0.07
D1466	0.14
D1466INT	0.05
D274	0.18
D274INT	0.05
D3128	0.06
D8880	0.11
ECOLI00	0.05
S. pullorum	0.01

Sample ID	S/P Ratio
ECOLI2	0.01
Fpox	0.01
IBD	0.08
ILT	0.01
ILTAGP	0.01
M41	0.08
M41INT	0.08
Mg	0.02
Ms	0.09
PMV1	0.09
PMV3	0.02
REO1133	0.02
REO2534	0.03
TRTA	0.09
TRTC	0.05

Sample ID	S/P Ratio
AI H7	3.04
AI H5	3.93
AI H6	3.81
AI H1 field L	2.08
AI H9 field R	2.1

DATA SHEETS

FIELD DATA

Flocks testing positive on the BioChek AI ELISA. All these cases were confirmed positive for Avian Influenza by HI in various laboratories.

		Page :	1	Date :	17-03-2003
	Assay : A1 Bleeding Date: 14-03-2003 Testing Date: 14-03-2003 #Samples: 19 Name: CHICKENS B5 POS Company: 1045 Code: Reason: SCREEN Complex: House:01 Type: Age:	Mean Titer: 4 265 G.M.T.: 3 073 %CV: 70			
	Comments Field infection, chickens positive on HI strain H5.				
	Assay : A1 Bleeding Date: 14-03-2003 Testing Date: 14-03-2003 #Samples: 5 Name: B-1 POS Company: LDU1 Code: Reason: B-1 POSITIVE Complex: House: Type: Age:	Mean Titer: 3 203 G.M.T.: 1 630 %CV: 76			
	Comments Screening with Bio Chek AI kit, positive samples confirmed on HI to be strain H1 (Deventer institute Holland)				
	Assay : A1 Bleeding Date: 14-03-2003 Testing Date: 14-03-2003 #Samples: 7 Name: B-6 POSITIVE Company: LDU1 Code: Reason: B-6 POS Complex: House: Type: Age:	Mean Titer: 2 767 G.M.T.: 2 635 %CV: 37			
	Comments Samples confirmed to be H6 positive by HI test.				
	Assay : A1 Bleeding Date: 14-03-2003 Testing Date: 14-03-2003 #Samples: 8 Name: TURKEYS B5 POS Company: 737 Code: Reason: SCREEN POS Complex: House:01 Type: T Age:	Mean Titer: 5 219 G.M.T.: 6 219 %CV: 56			
	Comments Field infection, Turkeys positive for H5 by HI test.				

DATA SHEETS

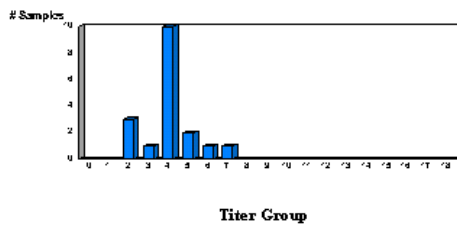
CASE STUDY

In Broilers, acute mortality is in excess of 30%. The majority of the symptoms are respiratory, congested lungs, inflammation of tracheas. No response to antibiotics. Serum samples were tested at BioChek for NDV, IBV, ART, Mg, Ms and AI. All results were as expected; only AI was strong positive in all flocks. Samples were sent to VLA for confirmation. Out of 40 positive samples sent, 11 samples tested positive for strain H9 using the HI test.



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Page : 1 Date : 17-03-2003

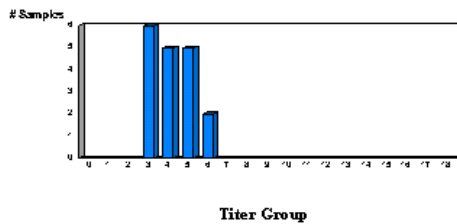


Assay: AI
Bleeding Date: 26-04-2002
Testing Date: 17-03-2003
#Samples: 18
Name: 1
Company: R
Code: 00119
Reason:
Complex: House: 31
Type: CM Age: 36D

Mean Titer	5 171
G.M.T.:	4 723
%CV :	39

Comments

Samples sent to VLA for HI on Avian Influenza. H9 positive

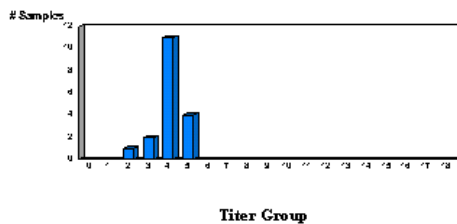


Assay: AI
Bleeding Date: 26-04-2002
Testing Date: 17-03-2003
#Samples: 18
Name: 2
Company: R
Code: 00119
Reason:
Complex: House: 32
Type: CM Age: 38D

Mean Titer	5 531
G.M.T.:	5 194
%CV :	37

Comments

Samples sent to VLA for HI on Avian Influenza. H9 positive



Assay: AI
Bleeding Date: 26-04-2002
Testing Date: 17-03-2003
#Samples: 18
Name: 3
Company: R
Code: 00119
Reason:
Complex: House: 33
Type: CM Age: 36D

Mean Titer	4 804
G.M.T.:	4 586
%CV :	27

Comments

Samples sent to VLA for HI on Avian Influenza. H9 positive

AVIAN INFLUENZA: AN OVERVIEW

Barend van Dam, BioChek 22/06/2000

Since the end of 1999 the North of Italy has been suffering from outbreaks with Highly Pathogenic Influenza (=HPAI) of the type H7N1. The IVPI (Intravenous Pathogenicity Index) has been determined to be 3, which means that the virus is extremely virulent and can cause mortality of 100%. So far 15 million poultry has either died or been culled. 50% of these problems were in Turkeys.

The virus:

Avian Influenza is caused by Influenza type A; it belongs to the group of Orthomyxoviridae. The Influenza virus is subtyped based on surface antigens H (Haemagglutinin) and N (Neuraminidase) there are 15 types of H, H1 – H15 and 9 types of N (N1-N9).

Pathogenicity

In the EEC HPAI is defined as following:

An infection of Poultry with a A-type influenza with a IVPI higher than 1.2 or an infection of Poultry with subtype H5 or H7 with a certain amino acid sequence. Therefore of every isolate of H5 or H7 the amino acid sequence needs to be determined.

Pathogenicity of the virus may change after passage in chickens. For example the outbreak in Pennsylvania in 1983 started with respiratory symptoms, a mortality of 0 – 15% and a decreased egg production. The virus was typed as H5N2. After half a year mortality suddenly increased to 80% with all signs of HPAI. The virus isolated was the same subtype H5N2. In Italy the same occurred, the outbreak started in March 1999 with symptoms of low pathogenic AI, H7N1 was isolated and classified as low pathogenic. In December 1999 the situation changed, symptoms of HPAI were seen and the subtype isolated, H7N1 was classified as HPAI.

Pathogenesis and symptoms

Symptoms will vary depending on subtype of AI and age of the birds etc. HPAI replicates in endothelium and therefore can affect all organs; low pathogenic AI will in general only replicate in the respiratory tract. The most obvious symptom is that the flock is quiet. In flocks in lay a drop in egg production often occurs.

Infections with HPAI will lead to severe disease due to hemorrhagic septicemia, after an incubation time of 1 – 7 days. Symptoms are ruffled feathers, no feed intake, high water consumption, strong drop in egg production and watery diarrhea. Mortality can suddenly increase, affected or dead chickens often have swollen combs and edema around the eyes. Cyanotic areas can be seen on the naked skin. The diarrhea will initially be watery, light green and will change to almost white. Surviving chickens will often have neurological signs. Mortality can reach 100%.

During the egg production moderate to severe drops in egg production can occur.

Post Mortem

Low pathogenic AI: Lesions limited to respiratory tract, tracheitis, bronchitis, pneumonia

HPAI: After acute death there often aren't any lesions, when death occurred less acutely lesions due to hemorrhagic septicemia will be seen. Micro bleedings (petechiae) will be found in larynx, trachea, proventriculus, pancreas and epicardial fat. On the mucous membranes, similar lesions can be found. General subcutaneous edema will be present. Yellow or grey necrotic lesions can be found on liver, spleen, kidneys and lungs. The carcass may show signs of dehydration.

Diagnosis

Differential diagnosis for Chickens: Newcastle Disease, Acute Fowl Cholera, other diseases causing septicemia, Infectious Laryngotracheitis & Infectious Bronchitis.

Virus isolation

In the acute phase cloacal- or tracheal swabs contain lots of virus. In a later stage affected organs are the most suitable sample. Samples for virus isolation should be transported cool.

Serology

AGP, HI and ELISA

AGP and ELISA are virus specific meaning that these will detect antibodies to type A influenza, all H and N types. HI will differentiate between the various H types (H1 – H15).

20 samples per flock is sufficient, as the virus spreads very fast.

After isolation and typing of the virus the virulence (Intravenous Pathogenicity Index) of the virus needs to be determined.

Prevention

Outbreaks caused by highly pathogenic influenza strains are being controlled by stamping out of the infected flocks and by restricting movement in affected areas. Vaccinations have so far not proven to be able to prevent the disease.

Eradication is focussed on the highly pathogenic strains, although one shouldn't ignore the strains with lower pathogenicity, as these are able to mutate to highly pathogenic strains. Therefore it's recommended by the scientific board of the European Committee to direct preventive measures at flocks infected with strains H5 and H7 of influenza viruses.

Preventive measures Holland

Definitions

Suspect flocks:

All poultry flocks for which the following applies

- Clinical symptoms
- Typical lesions in processing plant
- Serologically positive for AI strain H5 or H7
- Otherwise positive for AI strains H5 or H7

Positive flocks:

- All poultry flocks in which high pathogenic AI virus is demonstrated
- Highly pathogenic AI virus is virus with IVPI > 1.2 or H5 or H7 subtypes with an IVPI less than 1.2 but with a certain aminoacid sequence.

Strategy

- Trace suspect flocks
- Isolate suspect flocks
- Determine whether the suspect flocks are positive or not

Trace suspect flocks

Poultry farmers and veterinarians are obliged to report suspect flocks.

Isolate suspect flocks:

1. No movement of poultry in or out of the farm
2. No movement of other animals on the farm
3. No movement eggs, eggs for industrial use might get an exempt
4. No movement of vehicles from or to the farm
5. No movement of dead chickens, feed, meat or manure
6. No people are allowed into the houses
7. Extra disinfecting
8. In the case that manure is stored outside, treat with NaOH and cover
9. Have a good flock log book available
10. Visit of specialist team to determine if flock is positive

Determine if a flock is positive or not

For the first flock this must be done in a specialist lab, these will test samples and determine if the flock is infected with HPAI or not. This takes maximally 14 days. After the first flock has been declared positive, new flocks can be declared positive by a specialist team on clinical signs and post mortems.

When positive the following actions will be taken:

- Culling of the flock
- Rodent/insect killing
- Destruction of chickens and eggs
- Destruction of feed, manure and other organic materials possibly contaminated with AI virus
- Thorough disinfecting of poultry house
- Destruction of meat and eggs within incubation time of virus. Put already hatched chicks under surveillance.
- Repopulate no sooner than 21 days after culling.
- Install a 10 KM safety zone around positive farm.

Safety zone:

1. No transport of chickens
2. No transport of hatching eggs
3. No movement of poultry transport vehicles
4. No movement of poultry manure
5. No admission of outside people to farms
6. Thorough monitoring on poultry farms and serological monitoring
7. Proper disinfecting
8. No markets or shows
9. Backyard chickens in cages
10. These measures must be in place for at least 21 days after the last break, area will then be declared area with extra attention.

BioChek doesn't take any responsibility on action taken on basis of this information.