

Flu DETECT[®]

Avian Influenza Virus Type A Antigen Test Kit

The Synbiotics' Flu DETECT[®] Avian Influenza Virus Type A Antigen Test Kit is an in-vitro, rapid immunochromatographic assay which qualitatively detects Influenza Type A virus in tracheal and cloacal swabs from symptomatic birds or flocks. This test kit aids in the detection of all 16 subtypes of Influenza Type A virus. The kit contains swabs, test tubes, test strips and reagents necessary to test 20 samples. Simply insert a test strip into an extracted sample and read the results in 15 minutes!

Specificity

The results in Figure 1 below demonstrate that the Synbiotics' Flu DETECT[®] Avian Influenza Type A Antigen Test Kit shows excellent specificity to Influenza Type A virus. Like all Synbiotics' ELISA kits, this test is highly specific to provide valid reproducible results.

Figure 1: Subtype Reactivity

Influenza A Subtype Reactivity

A/New Caledonia/20/99 (H1N1)	Positive
A/Swine/TN/25/77 (H1N1)	Positive
A/Mallard/Alberta/11(9/98) (H1N1)	Positive
VR-897, A/New Jersey/8/76 (H1N1)	Positive
VR-97, A/FM/1/47 (H1N1)	Positive
VR-98, A/Malaya/302/54 (H1N1)	Positive
VR-95, A/PR/8/34 (H1N1)	Positive
A/New Caledonia/20/99 (H1N1)	Positive
A/Taiwan/1/86 (H1N1)	Positive
A/Beijing/262/95 (H1N1)	Positive
A/Mallard/NY/6750/78 (H2N2)	Positive
A/Mild/Potsdm/178-4/83 (H2N2)	Positive
VR-100, A2/Japan/305/57 (H2N2)	Positive
A/Mallard/Alberta/33/01 (H2N4)	Positive
A/Ruddy Turnstone/DE/142/99 (H3N2)	Positive
A/HongKong/1/68 (H3N2)	Positive
A/Duck/HK/3/75 (H3N2)	Positive
A/HK/1/68/Luman (H3N2)	Positive
VR-810, A/Port Chalmers/73 (H3N2)	Positive
VR-544, A/Hong Kong/8/68 (H3N2)	Positive
VR-547, A/Aichi,2/68 (H3N2)	Positive
VR-822, A/Victoria/3/75 (H3N2)	Positive
A/Panama/2007/99 (H3N2)	Positive
A/Kiev/301/94 (H3N2)	Positive
A/Shangdong/9/93 (H3N2)	Positive
A/Texas/1/77 (H3N2)	Positive
A/Mallard/Alberta/31/01 (H3N9)	Positive
CK/AI/75/A (H4N8)	Positive
Duck Meat/AVL/China-1/01 (H5N1)	Positive
AI/Chicken/86 (H5N2)	Positive
A/Teal/HongKong/w312/97 (H6N1)	Positive
A/Mallard/Alberta/20 (6/96) (H6N8)	Positive

A/CK/DE/04/297267(Hobo Farm) (H7N2)	Positive
A/Mild/Alberta/24/01 (H7N3)	Positive
A/Mallard/Alberta/19 (4/92) (H8N4)	Positive
A/Qa/AR/29209-1 (H9N2)	Positive
A/Ck/HK/WF2/99 (H9N2)	Positive
A/Ck/HK/SF3/99 (H9N2)	Positive
A/Ck/HK/G9/97 (H9N2)	Positive
A/Duck/HK/Y280/97 (H9N2)	Positive
RG A/Qa/HK/A28945/88(H9N2) 10/16/03A/RG (H9N2)	Positive
A/Shorebird/DE (9/96) (H9N6)	Positive
A/Pintail/Alberta/202/00 (H10N7)	Positive

Influenza B and non-Influenza Reactivity

VR-790, Russia/69	Negative
VR-295, B/Taiwan/2/62	Negative
VR-102, B/Allen/45	Negative
B/Tokio/53/99	Negative
VR-101, B/Lee/40	Negative
B/Victoria/504/00	Negative
B/Quingdao/102/91	Negative
VR-823, B/Hong Kong/5/72	Negative
CAP Flu B VR1-04 2004, B/Maryland/1/59	Negative
RSV VR-1341, Caprine, Lot 1W	Negative
RSV VR-1401, B, Wash/18537	Negative
RSV Strain A-2, Lot 263504	Negative
RSV VR-1400, B-1, Wild Type	Negative
RSV VR-26	Negative
Adenovirus 8, 9/23/94	Negative
Adenovirus 40, VR-931, Lot 6W	Negative
Adenovirus 11, TH408	Negative
Adenovirus 2, TJ2184	Negative

Specificity

The data in Figure 2 below exhibits specificity as compared to a competitor's rapid test. In this study various non-AIV antigen preparations were tested along with a positive control containing H4N8, CK/AI/75/A ($10^{7.9}/0.1$ mL).

Figure 2: Specificity

	SBIO H4N8	Kit A H4N8	
A	Negative	Negative	A = NDV CEK Primary Cells, first passage (tissue pool of trachea, eyelid)
B	Negative	Negative	B = NDV 3rd passage in eggs (tissue pool of trachea, lungs)
C	Negative	Negative	C = ILT 1st passage in eggs (tissue pool of trachea, eyelid)
D	Negative	Negative	D = ILT 1st passage in eggs (tissue pool of trachea, eyelid)
E	Negative	Negative	E = NDV CEK Primary Cells, second passage (tissue pool of trachea)
F	Negative	Negative	F = ILT CEK Primary Cells, first passage (tissue pool of trachea, eyelid)
G	Negative	Negative	G = REO CEK Primary Cells, 3rd passage (spleen)
H	Negative	Negative	H = NDV 3rd passage in eggs (tissue pool of trachea, lungs)
I	Negative	Positive	I = Feces spiked with B
J	Negative	Positive	J = Feces spiked with G
K	Negative	Positive	K = Feces spiked with F
L	Positive	Positive	L = Feces spiked with positive control (H4N8, CK/AI/75/A)

Sensitivity

The data in Figure 3 demonstrate the sensitivity of detecting Type A AIV. In this study, quail were infected with H9N2 virus, tracheal swabs were collected and results were compared to Virus Isolation as shown below.

Figure 3: Sensitivity comparison with Virus Isolation

Day	SBIO	Virus Isolation
0	0/10	0/10
1	7/10	Not Tested
3	9/10	10/10
5	10/10	10/10
7	4/10	10/10
9	0/10	6/10
11	0/10	0/10

Based upon these data, the correlation with the VI is 90-100% during the first 3 to 5 days post infection.

These data indicate that Flu DETECT® is efficacious for the surveillance of early Avian Influenza infection.

Figure 4: Sensitivity comparison with Competitors

Figure 4 below provides data comparing the sensitivity of Flu DETECT® to competitors' rapid tests.

H4N8 CK/AI/75/A ($10^{8.9}/\text{mL}$)			H7N2 A/CK/DE/04/297267 ($8 \times 10^9 \text{EID}_{50}/\text{mL}$)			H5N2 AI/Chicken/86		H7N2 A/Mallard/NY/6750/78 ($5 \times 10^7 \text{EID}_{50}/\text{mL}$)		
	SBIO	Kit A	SBIO	Kit A	Kit B	Kit A	Kit B	SBIO	Kit A	Kit B
10^{-1}	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
10^{-2}	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
10^{-3}	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Negative
10^{-4}	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Positive	Negative	Negative
10^{-5}	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Negative
10^{-6}	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative
10^{-7}	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	N/A
10^{-8}	Negative	Negative	Negative	Negative	Negative	N/A	N/A	N/A	N/A	N/A
10^{-9}	Negative	Negative	Negative	Negative	Negative					
10^{-10}	Negative	Negative	N/A	N/A	N/A					
10^{-11}	Negative	Negative								
10^{-12}	Negative	Negative								

Based upon these results and other testing, the minimum detectability of the Synbiotics' Flu DETECT® Avian Influenza Virus Type A Antigen Test Kit is determined to be $10^3 - 10^4$ EID₅₀/mL.



12200 N. Ambassador Drive, Suite 101 • Kansas City MO 64163
800-228-4305 • 816-464-3500 • Fax: 816-464-3521
www.synbiotics.com

Synbiotics France
2, rue Alexander Fleming • 69367 LYON Cedex 07
+33 4 72 76 11 11 • Fax: +33 4 72 76 11 10
www.synbiotics.fr