

# Evaluation of the BD Directigen™ EZ Flu A+B Rapid Chromatographic Immunoassay\*

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## ABSTRACT

**BACKGROUND:** The BD Directigen™ EZ Flu A+B test is a rapid chromatographic immunoassay for the direct, qualitative and separate detection of influenza A and B virus from a single processed specimen using a single device.

**OBJECTIVES:** To compare the performance of the BD Directigen™ EZ Flu A+B (BD) test with other commercially available rapid influenza tests: BinaxNOW® Influenza A&B (BN), Remel Xpect™ Flu A&B (RX), Quidel QuickVue® Influenza A+B (QQ); and to evaluate the ability of the BD test to detect both the Avian Flu and California Flu strains.

**METHODS AND RESULTS:** Comparative Limit of Detection (LOD) Study: Serial dilutions of influenza A and B viral isolates were tested to determine the lowest level of virus resulting in positive results for each kit.

Results for the Comparative LOD (TCID<sub>50</sub>/mL) Study

	BD	BN	RX	QQ
Influenza A	2.5 x 10 <sup>3</sup>	1.0 x 10 <sup>4</sup>	1.0 x 10 <sup>4</sup>	5.0 x 10 <sup>3</sup>
Influenza B	1.0 x 10 <sup>2</sup>	1.0 x 10 <sup>2</sup>	2.0 x 10 <sup>2</sup>	5.0 x 10 <sup>1</sup>

**Seeded Specimen Study:** Thirty surplus clinical nasal aspirate specimens, consisting of 10 negatives, 10 influenza B weak positives (negative specimens seeded with influenza B virus at 2 x 10<sup>2</sup> TCID<sub>50</sub>/mL), and 10 influenza A weak positives (5 negative specimens seeded with influenza A virus at 5 x 10<sup>3</sup> TCID<sub>50</sub>/mL and 5 influenza A strong positive specimens diluted with negative specimen) were tested in a randomized, blinded fashion.

Results for the Seeded Specimen Study

	BD	BN	RX	QQ
Influenza A Positive	10/10	3/10	5/10	9/10
Influenza B Positive	9/9**	10/10	10/10	10/10
Influenza Negative	10/10	9/10	8/10	8/10

**Strain Reactivity Study:** The BD test was evaluated using a panel of 23\*\*\* influenza A and 11 influenza B viral isolates in a randomized, blinded fashion. The BD test specifically recognized all influenza A and influenza B samples tested, and recognized all 15 hemagglutinin and 9 neuraminidase subtypes of influenza A. The BD test also was able to detect three human influenza A H5N1 (avian influenza) isolates, as well as the A/California/07/2004 strain (recommended by WHO for use in the 2005-2006 vaccine).

**CONCLUSIONS:** The BD Directigen™ EZ Flu A+B test:

- had the lowest LOD on influenza A compared to the other evaluated influenza tests and had equal or better LOD on influenza B compared to the Remel and Binax Flu kits.
- was more sensitive in detecting influenza A than the Binax and Remel Flu kits on weak positive specimens.
- accurately detected all 15 hemagglutinin and 9 neuraminidase subtypes of influenza A strains including 3 avian influenza (H5N1) isolates and the A/California/07/2004 strain.

## INTRODUCTION

Influenza is an acute viral disease that is seasonal in incidence. Influenza A or B viruses cause the majority of clinically significant disease. Since the therapeutic options have expanded to include options for the treatment of both influenza A and influenza B disease, it is important to rapidly distinguish influenza A from influenza B in order to allow physicians a choice in selective antiviral intervention.

The BD Directigen™ EZ Flu A+B antigen detection test is a chromatographic immunoassay to detect influenza A or B antigens extracted from respiratory specimens of symptomatic patients. Assay test time is 15 minutes with reactivity determined by the appearance of a reddish-purple line.

The speed and simplified workflow of the BD Directigen™ EZ Flu A+B test make it applicable as a “STAT” influenza A and B antigen detection test, providing rapid, relevant information to assist with the diagnosis of influenza. The use of the BD Directigen™ EZ Flu A+B test to differentiate Flu A from Flu B infection can provide the opportunity for greater selectivity of antiviral intervention.



\* Pending FDA Clearance.  
\*\* One uninterpretable specimen.  
\*\*\* The total number of influenza A strains were actually 26.

## MATERIALS AND METHODS

### MATERIALS

**Viruses:** Flu A/PR/8/64 (TCID<sub>50</sub>/mL = 5 x 10<sup>6</sup>/mL) and Flu B/Lee/40 (TCID<sub>50</sub>/mL = 5 x 10<sup>4</sup>/mL) viruses used in the comparative LOD study and seeded specimen study were prepared at BD. The Flu A/California/07/04 virus was obtained from Dr. Alexander Klimov's lab at the CDC. Viral counts were determined by ABI (Advance Biotechnologies Inc.).

A panel of 23 influenza A viruses (including 15 animal strains representing 15 hemagglutinin and 9 neuraminidase subtypes obtained from Dr Swayne's laboratory at the USDA, 7 human strains obtained from American Type Culture Collection and 1 human strain obtained from Dr. Alexander Klimov's lab at the CDC) and 11 human influenza B viruses (obtained from ATCC and other sources) was used for the strain reactivity study.

**Clinical specimens:** All clinical surplus specimens used in the seeded specimen study were frozen nasal aspirates obtained from Children's Hospital of Buffalo.

#### Test Kits:

BD Directigen™ EZ Flu A+B  
(BD Diagnostic Systems, Sparks MD) – (BD)

Binax NOW® Influenza A&B  
(Binax, Inc., Portland ME) – (BN)

Remel Xpect™ Flu A&B  
(Remel, Lenexa, KS) – (RX)

Quidel QuickVue® Influenza A+B  
(Quidel, San Diego, CA) – (QQ).

#### PBS:

Gibco/Invitrogen DPBS (for making virus dilutions).

### METHODS

#### I. Comparative Limit of Detection (LOD) Study:

Two-fold serial dilutions of Flu A/PR/8/64, Flu A/Cal/07/04 and Flu B/Lee/40 viruses were prepared in PBS to result in six concentration levels, such that an assay endpoint was achieved and at least one dilution gave a negative test result for all three replicates on the BD Directigen™ EZ Flu A+B test. These six dilutions were then tested in triplicate in a randomized and blinded fashion on each of the four test kits to determine the lowest concentration of virus resulting in positive results for each kit. Assay for each kit was performed, and results interpreted according to the product insert for the respective kit.

#### Comparative Limit of Detection with Flu A/PR/8/64

Viral Concentration (TCID <sub>50</sub> /mL)	BD Directigen™ EZ Flu A+B	Binax NOW Flu A&B	Remel Xpect Flu A/B	Quidel QuickVue Flu A+B
1 x 10 <sup>4</sup>	P	WP	P	P
	P	WP	WP	P
	P	WP	P	P
5 x 10 <sup>3</sup>	P	N	U	P
	P	N	P	P
	P	N	N	P
2.5x10 <sup>3</sup>	P	N	N	N
	P	N	N	WP
	P	N	N	P
1.25 x 10 <sup>3</sup>	WP	N	N	N
	N	N	N	N
	N	N	N	WP
0.625 x 10 <sup>3</sup>	N	N	N	N
	N	N	N	N
	N	N	N	N
DPBS	N	N	N	N
	N	N	N	N
	N	N	N	N

#### Comparative Limit of Detection with Flu A/CAL/07/04

Viral Dilution	BD Directigen™ EZ Flu A+B	Binax NOW Flu A&B	Remel Xpect Flu A/B	Quidel QuickVue Flu A+B
1:800	P	P	P	P
	P	P	N	P
	P	P	N	P
1:1,600	P	N	N	P
	P	N	N	P
	P	N	N	P
1:3,200	P	N	N	N
	P	N	N	P
	N	N	N	N
1:6,400	N	N	N	N
	N	N	N	N
	P	N	N	N
1:12,800	N	N	N	P
	N	N	N	N
	N	N	N	N
DPBS	N	N	N	N
	N	N	N	N
	N	N	N	N

#### Comparative Limit of Detection with Flu B/LEE/40

Viral Concentration (TCID <sub>50</sub> /mL)	BD Directigen™ EZ Flu A+B	Binax NOW Flu A&B	Remel Xpect Flu A/B	Quidel QuickVue Flu A+B
8 x 10 <sup>2</sup>	P	P	P	P
	P	P	P	P
	P	P	P	P
4 x 10 <sup>2</sup>	P	P	P	P
	P	P	P	P
	P	P	P	P
2 x 10 <sup>2</sup>	P	P	P	P
	P	P	P	P
	P	P	P	P
1 x 10 <sup>2</sup>	P	WP	N	P
	P	WP	N	P
	P	WP	N	P
50	WP	N	N	P
	N	N	N	P
	WP	N	N	P
DPBS	N	N	N	N
	N	N	N	N
	N	N	N	N

NOTE: P = Positive; WP = Weak Positive; N = Negative; U = Uninterpretable  
Shaded areas indicate result at LOD. Any visible line was recorded as positive (P).  
Results with faint visible lines were recorded as weak positives (WP).

#### Summary of Results for the Comparative LOD (TCID<sub>50</sub>/mL) Study

	BD Directigen™ EZ Flu A+B	Binax NOW Flu A&B	Remel Xpect Flu A/B	Quidel QuickVue Flu A+B
Influenza A/PR/8/64	2.5 x 10 <sup>3</sup>	1.0 x 10 <sup>4</sup>	1.0 x 10 <sup>4</sup>	5.0 x 10 <sup>3</sup>
Influenza A/Cal/07/04	1:1,600	1:800	>1:800	1:1,600
Influenza B/Lee/40	1.0 x 10 <sup>2</sup>	1.0 x 10 <sup>2</sup>	2.0 x 10 <sup>2</sup>	5.0 x 10 <sup>1</sup>

## MATERIALS AND METHODS

### II. Seeded Specimen Study:

The four products were evaluated with thirty surplus clinical nasal aspirate specimens. A limited number of flu A positive samples were available and no flu B positive samples were available. The sample population was supplemented by seeding influenza negative samples with virus.

#### Sample Panel:

- 10 negatives aspirates (N1–N10)
- 10 influenza B weak positives (B1–B10) (negative aspirate specimens seeded with influenza B/Lee/40 virus at  $2 \times 10^2$  TCID<sub>50</sub>/mL)
- 10 influenza A weak positives [5 negative aspirate specimens (A1-A5) seeded with influenza A/PR/8/64 virus at  $5 \times 10^3$  TCID<sub>50</sub>/mL and 5 influenza A strong positive aspirate specimens (A6-A10) diluted with negative aspirate specimen]

Testing was conducted in a randomized, blinded fashion. Assay for each kit was performed and results interpreted according to the product insert for the respective kit.

### Seeded Specimen Study

#### Flu A Result:

Sample	BD Directigen™ EZ Flu A+B	Binax NOW Flu A&B	Remel Xpect Flu A/B	Quidel QuickVue Flu A+B
A1	P	N	N	P
A2	P	N	N	WP
A3	P	WP	P	WP
A4	P	N	N	P
A5	P	N	N	N
A6	P	WP	P	P
A7	P	N	N	P
A8	P	N	P	P
A9	P	N	P	P
A10	P	WP	P	P
B1	N	N	N	N
B2	U	N	N	N
B3	N	N	N	N
B4	N	N	N	N
B5	N	N	N	N
B6	N	N	N	N
B7	N	N	N	N
B8	N	N	N	N
B9	N	N	N	N
B10	N	N	N	N
N1	N	N	N	P
N2	N	N	N	N
N3	N	N	N	N
N4	N	N	N	N
N5	N	N	WP	N
N6	N	N	N	WP
N7	N	N	P	N
N8	N	N	N	N
N9	N	N	N	N
N10	N	N	N	N

#### Flu B Result:

Sample	BD Directigen™ EZ Flu A+B	Binax NOW Flu A&B	Remel Xpect Flu A/B	Quidel QuickVue Flu A+B
A1	N	N	N	N
A2	N	N	N	N
A3	N	N	N	N
A4	N	N	N	N
A5	N	N	N	N
A6	N	N	N	N
A7	N	N	N	N
A8	N	N	N	N
A9	N	N	N	N
A10	N	N	N	N
B1	P	P	P	P
B2	U	P	P	P
B3	P	P	P	P
B4	P	P	P	P
B5	P	P	P	P
B6	P	P	P	P
B7	P	P	P	P
B8	P	P	P	P
B9	P	P	P	P
B10	P	P	P	P
N1	N	N	N	N
N2	N	N	N	N
N3	N	N	N	N
N4	N	P	N	N
N5	N	N	N	N
N6	N	N	N	N
N7	N	N	N	N
N8	N	N	N	N
N9	N	N	N	N
N10	N	N	N	N

Note: A1-A10 = Flu A positive samples; B1-B10 = Flu B positive samples; N1-N10 = Negative samples  
P = Positive; WP = Weak Positive; N = Negative; U = Uninterpretable  
Shaded areas represent false negative, false positive or uninterpretable result.

#### Summary of Results for the Seeded Specimen Study

Samples	BD Directigen™ EZ Flu A+B	Binax NOW Flu A&B	Remel Xpect Flu A/B	Quide Quick Vue Flu A+B
Influenza A Positives	10/10	3/10	5/10	9/10
Influenza B Positives	9/9**	10/10	10/10	10/10
Influenza Negatives	10/10	9/10	8/10	8/10

\*\* One uninterpretable specimen.

## MATERIALS AND METHODS (continued)

### III. Strain Reactivity Study:

The BD Directigen™ EZ Flu A+B test was evaluated using a panel of 26 influenza A and 11 influenza B viral isolates in a randomized, blinded fashion. The BD test specifically recognized all influenza A and influenza B samples tested, and recognized all 15 hemagglutinin and 9 neuraminidase subtypes of influenza A. The BD test also was able to detect three human influenza A H5N1 (avian influenza) isolates, as well as the A/California/07/2004 strain (recommended by WHO for use in the 2005-2006 vaccine).

Table 1: Reactivity and Specificity of Human Strains

Influenza A Virus Human Strains	Viral Type	Tested Concentration	Flu A Result	Flu B Result
A/NWS/33	A (H1N1)	1.58E+06 CEID <sub>50</sub> /mL	P	N
A/PR/8/34	A (H1N1)	8.89E+07 CEID <sub>50</sub> /mL	P	N
A1/FM/1/47	A (H1N1)	1.58E+05 CEID <sub>50</sub> /mL	P	N
A1/Denver/1/57	A (H1N1)	8.89E+06 CEID <sub>50</sub> /mL	P	N
A/New Jersey/8/76 (Hsw N1)	A (H1N1)	8.89E+05 CEID <sub>50</sub> /mL	P	N
A/Port Chalmers/1/73	A (H3N2)	1.58E+05 CEID <sub>50</sub> /mL	P	N
A/Victoria/3/75	A (H3N2)	8.89E+07 CEID <sub>50</sub> /mL	P	N
A/California/07/04	A (H3N2)	120 HA	P	N
A/Vietnam/3028/04	A (H5N1)	4.7E+10 TCID <sub>50</sub> /mL	P	N
A/Thailand/MK2/04	A (H5N1)	5.2E+10 TCID <sub>50</sub> /mL	P	N
A/Hong Kong/486/97	A (H5N1)	Titer unknown	P	N

Table 2: Reactivity and Specificity of Human Flu B Strains

Influenza B Virus Human Strains	Viral Type	Tested Concentration	Flu A Result	Flu B Result
B/Lee/40	B	3.16E+08 CEID <sub>50</sub> /mL	N	P
B/Allen/45	B	1.58E+05 CEID <sub>50</sub> /mL	N	P
B/GL/1739/54	B	8.89E+05 CEID <sub>50</sub> /mL	N	P
B/Maryland/1/59	B	5.00E+05 CEID <sub>50</sub> /mL	N	P
B/Taiwan/2/62	B	2.81E+04 CEID <sub>50</sub> /mL	N	P
B/Mass/3/66	B	8.89E+03 CEID <sub>50</sub> /mL	N	P
B/Hong Kong/5/72	B	5.00E+08 CEID <sub>50</sub> /mL	N	P
B/Victoria/504/00	B	0.045 mg/300 µL	N	P
B/Tokyo/53/99	B	0.03 mg/300 µL	N	P
B/Quingdao/102/91	B	0.0345 mg/300 µL	N	P
B/Leningrad/86/93	B	0.0345 mg/300 µL	N	P

Table 3: Reactivity and Specificity of Animal Strains

Influenza Virus Animal Isolates	Sub Type	Tested Concentration (CEID <sub>50</sub> /mL)	Flu A Result	Flu B Result
A/Turkey/Kansas/4880/80	A (H1N1)	2.00E+08	P	N
A/Mallard/New York/6750/78	A (H2N2)	3.16E+07	P	N
A/Turkey/England/69	A (H3N2)	1.26E+07	P	N
A/Chicken/Alabama/75	A (H4N8)	2.00E+06	P	N
A/Turkey/Wisconsin/68	A (H5N9)	7.94E+07	P	N
A/Turkey/Canada/63	A (H6N8)	7.94E+05	P	N
A/Turkey/Oregon/71	A (H7N3)	1.26E+07	P	N
A/Turkey/Ontario/6118/67	A (H8N4)	1.26E+07	P	N
A/Turkey/Wisconsin/66	A (H9N2)	2.00E+06	P	N
A/Chicken/Germany/N/49	A (H10N7)	5.01E+07	P	N
A/Duck/Memphis/546/74	A (H11N9)	7.94E+07	P	N
A/Duck/Alberta/60/76	A (H12N5)	3.16E+07	P	N
A/Gull/MD/704/77	A (H13N6)	3.16E+07	P	N
A/Mallard/Gurjev/263/82	A (H14N5)	3.16E+07	P	N
A/Shearwater/WA/2576/79	A (H15N6)	2.00E+06	P	N

P = Positive  
N = Negative

## DISCUSSION

### I. Comparative LOD Study:

- The BD Directigen™ EZ Flu A+B test detected the lowest concentration of virus (LOD) with influenza A/PR/8/64 and A/California/07/04 compared to the other evaluated influenza tests.
- The BD test detected equal to or lower concentration of virus (LOD) with influenza B/Lee/40 compared to the Binax and Remel tests.

### II. Seeded Specimen Study:

- The BD Directigen™ EZ Flu A+B test detected more weak positive (10/10 or 100%) influenza A samples than the Binax and Remel Flu tests on weak flu A positive specimens. Although the Quidel test was almost as sensitive as the BD test, the results for two of the specimens were weak positives.
- The four tests showed equal performance in detecting weak flu B positive specimens.

### III. Strain Reactivity and Specificity Study:

- The BD Directigen™ EZ Flu A+B test accurately detected all 15 hemagglutinin and 9 neuraminidase subtypes of influenza A strains.
- All of the influenza A viruses tested gave positive flu A test results and negative flu B test results. All of the influenza B viruses tested gave positive flu B test results and negative flu A test results. This study showed that the BD Directigen™ EZ Flu A+B test accurately and specifically detects a diverse population of flu A and B strains.
- There is a concern about a potential outbreak of the deadly avian flu A (H5N1) virus. WHO has also recommended the use of the flu A/California/07/04 strain for the first time in the 2005-2006 vaccines. The BD Directigen™ EZ Flu A+B test can detect both of these strains of influenza A virus.

The speed and simple workflow of the BD Directigen™ EZ Flu A+B test make it applicable as a “STAT” influenza A and B antigen detection test, providing rapid, relevant information to assist the diagnosis of influenza. The BD Directigen™ EZ Flu A+B test can accurately detect flu A and flu B viral antigens including the new A/California(H2N3) and the avian (H5N1) strains.