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**x/pect™ FLU A&B****INTENDED USE**

REMEl's Xpect™ Flu A&B is a rapid *in vitro* immunochromatographic test for the direct, qualitative detection of influenza A and influenza B viral antigen (nucleoprotein) from nasal wash, nasal swab, and throat swab specimens from symptomatic patients. The test is intended as an aid in the rapid diagnosis of influenza A and influenza B viral infections. Negative tests should be confirmed by cell culture.

**SUMMARY AND EXPLANATION**

Influenza is a highly contagious, acute respiratory illness characterized by the abrupt onset of fever, myalgia, headache, malaise, nonproductive cough, sore throat, and rhinitis. Worldwide, the occurrence of influenza follows seasonal patterns unique to the geographical area, but with international travel the disease may become more of a year-round phenomenon. Influenza type A and B viruses are responsible for the epidemics of disease that occur almost every winter.<sup>1</sup> In the United States, these winter influenza epidemics can cause illness in 10-20% of the population and are associated with an average of 36,000 deaths and 114,000 hospitalizations per year.<sup>2</sup> Influenza type C is usually associated with only mild or asymptomatic disease.<sup>3</sup>

Influenza typically resolves in one or two weeks, but some people will suffer life-threatening complications. Influenza can exacerbate underlying medical conditions (e.g. cardiopulmonary conditions), lead to secondary bacterial pneumonia or primary influenza viral pneumonia, or occur as part of a co-infection with other viral or bacterial pathogens. Pneumonia and influenza together are the seventh most common cause of death in the United States and the fifth leading cause of death among all Americans over the age of 65.<sup>4</sup>

The appropriate treatment of patients with influenza-like illness depends on accurate and timely diagnosis, which can help reduce the inappropriate use of antibiotics and provide the option of using antiviral therapy. Diagnosis of influenza based on symptoms alone is difficult because the initial symptoms can be similar to those caused by other infectious agents. A variety of laboratory methods are available for the detection of influenza virus including conventional cell culture, shell vial culture, immunofluorescent staining, serologic tests, amplified nucleic acid detection assays, and rapid immunoassays.<sup>5,6,7</sup> If diagnosed within 48 hours of the onset of symptoms, several antiviral medications are available which may shorten the duration and lessen the severity of symptoms associated with uncomplicated influenza illness. These medications can also be used as prophylaxis to help prevent disease in patient contacts. Amantadine and rimantadine are available for preventing or treating influenza A only. The neuraminidase inhibitor class of antiviral drugs for influenza (zanamivir and oseltamivir) is effective in preventing and treating both types A and B. Because the drugs differ in their route of administration, dosage regimen, side effects, indications for use (patient age), and cost, it is useful to distinguish between influenza A and influenza B infections to make the most appropriate therapeutic decision.

The Xpect™ Flu A&B provides valuable, practical information to aid in the diagnosis of influenza in symptomatic patients. Physicians can quickly identify those patients who will benefit from treatment and establish the proper treatment regimen, which is essential in effectively controlling and preventing influenza.

**PRINCIPLE**

The Xpect™ Flu A&B is a chromatographic immunoassay for the qualitative detection of influenza A and influenza B viral antigens. The test device incorporates separate membrane strips for influenza A and for influenza B. To perform the test, the patient specimen is diluted and added to the sample well of the device. The mixture moves along the membranes by capillary action. If present, influenza A or B viral antigens in the patient sample bind anti-influenza A or B conjugated antibodies. A visible line forms as a complex of antibody-antigen-antibody coated colored particles is captured in the test region (T). Antibody coated colored particles not bound at the test line are later captured in the control region (C) containing goat anti-mouse antibody. A visible line will always appear in the control region indicating that the test is working properly. The presence of a control line combined with the absence of a visible test line is interpreted as a negative test result.

**STORAGE**

The product should be stored at room temperature (20-25°C) or refrigerated (2-8°C) until the expiration date printed on the box. Do not freeze or overheat. If stored refrigerated, allow components to come to room temperature before use.

**PRECAUTIONS**

- For *In Vitro* Diagnostic Use Only.
- Directions should be read and followed carefully.
- Do not use components beyond the printed expiration dates.
- Do not reuse kit components or test devices.
- Only rayon or dacron-tipped swabs with aluminum or plastic shafts should be used with this test. Calcium alginate swabs should not be used.
- Follow established laboratory safety procedures when working with patient specimens.
- Standard precautions should be taken against the dangers of biological hazards by properly sterilizing specimens, containers, and test devices after their use.

**SPECIMEN COLLECTION, STORAGE AND TRANSPORTATION**

Acceptable specimens for evaluation with the Xpect™ Flu A&B test include nasal washes, nasal swabs, and throat swabs. It is recommended that specimens be obtained early in the course of the illness and be tested as soon as possible. Freshly collected specimens may be run immediately without the use of transport medium. Alternatively, samples can be placed into a suitable transport medium, maintained at 2-8°C and tested within 72 hours after collection. Frozen specimens in a suitable liquid viral transport medium stored at -20°C or below in a non-defrosting freezer can be tested up to six months after collection. Avoid multiple freeze-thaw cycles.

**Transport Media:**

The following transport media have been evaluated and found to be compatible with Xpect™ Flu A&B test.

Amies Medium  
Bartels Viral Transport Medium  
Cary Blair Medium  
Earle's Minimum Essential Medium (EMEM)  
EMEM with 1% Bovine Serum Albumin  
EMEM with 1% Lactalbumin Hydrolysate  
Hank's Balanced Salt Solution  
Liquid Stuarts Medium  
M4™  
M4-RT™  
M5™  
Phosphate Buffered Saline (PBS)  
PBS with 0.5% Bovine Serum Albumin

- PBS with 0.5% Gelatin
- Saline (normal)
- Sucrose Phosphate
- Tryptic Soy Broth with 0.5% Bovine Serum Albumin
- Tryptic Soy Broth with 0.5% Gelatin
- Veal Infusion Broth
- Veal Infusion Broth with 0.5% Bovine Serum Albumin

**REAGENTS AND MATERIALS SUPPLIED**

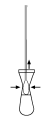
1. **Test devices (20):** Each foil pouch contains one single-use test device with two membrane strips. The strips in the device contain antibodies to influenza A or B.
2. **Specimen Diluent (20 ml):** One dropper bottle containing a buffered saline solution with detergent, a mucolytic agent, and preservative.
3. **Dilution tubes (20):** Flexible plastic tubes for specimen preparation.
4. **Disposable transfer pipettes (20):** Pipettes with marked graduations at approximately 0.1 ml increments.
5. **Flu A (+)/B (-) control swab (1):** A dry swab containing inactivated influenza A antigen.
6. **Flu B (+)/A (-) control swab (1):** A dry swab containing inactivated influenza B antigen.
7. **Instructions for Use (1).**

**MATERIALS REQUIRED BUT NOT SUPPLIED**

1. Timer
2. Specimen Collection Containers and Transport Media

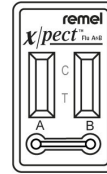
**PROCEDURE**

1. Remove the test device from the foil pouch when ready to perform the test and place it on a flat surface. (Allow kit components to equilibrate to room temperature if stored refrigerated.)
  2. Label the device with patient or control identification.
  3. Sample preparation:
    - a. For **swab specimens without dilution\* in transport media** (including the Quality Control Swabs provided with the kit):
      - i. Dispense 25 drops (approximately 0.6 ml) of Specimen Diluent into a dilution tube.
      - ii. Place the swab specimen in the tube.
      - iii. Mix thoroughly or vortex to release bound antigenic material from the swab.
      - iv. Rotate the swab firmly against the tube walls then squeeze the sides of the tube (as depicted) while removing the swab.
- \*Swab not submerged in a sufficient volume of transport media to allow processing of 0.1 ml.*
- b. For **all specimens except swab specimens without transport media:**
    - i. Dispense 5 drops (approximately 0.1 ml) of Specimen Diluent into the dilution tube provided.
    - ii. Mix specimen well and use the transfer pipette provided in the kit to transfer 0.1 ml (first molded graduated mark from the tip) of liquid specimen (nasal wash or specimens in transport medium) into the dilution tube provided.
  4. Use a transfer pipette to dispense 0.2 ml (2<sup>nd</sup> graduated mark from tip of pipette) of specimen into the center of the sample well of the test device.
  5. Read and record the test results visually after 15 minutes (or up to 30 minutes) according to the INTERPRETATION section. (Strong positive results may be apparent sooner than 15 minutes.)



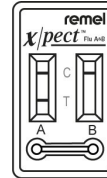
**INTERPRETATION**

The test device has two separate read windows; the one on the left is for Flu A and the one on the right is for Flu B as depicted.



**Positive Test (antigen present):**

A positive test is indicated by two black-colored bands; one in the (T) region and one in the (C) region.



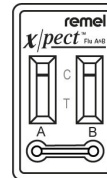
Flu A positive



Flu B positive

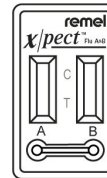
**Negative Test (antigen not detected):**

A negative test is indicated by only one black-colored band in the control (C) region.



**Invalid Test:**

No black-colored band in the control (C) region. A complete, black, clearly visible test line of any intensity should be interpreted as positive. Invalid results due to excessively mucoid specimens may be repeated using twice the normal volume of Specimen Diluent during the dilution step.



Uninterpretable

**Reporting Results:**

It is recommended that results be reported as follows:

- |          |   |
|----------|---|
| Positive | Positive for influenza A and/or influenza B antigen.  |
| Negative | Negative for influenza A and/or influenza B antigen. Infection due to influenza A or B cannot be ruled out since the antigen present in the specimen may be below the detection limit of the test. Culture confirmation of negative samples is recommended. |

**QUALITY CONTROL**

**Internal:** A procedural control is included in the test. A colored band appearing on the control band (C) region is considered an internal positive procedural control, indicating proper performance and reactive reagents. A clear background in the results area is considered an internal negative control. If the test has been performed correctly and reagents are working properly, the background will clear to give a discernible result.

**External:** It is recommended that Positive and Negative controls be run with each new test kit lot number. Each laboratory should follow their state and local requirements.

Quality Control swabs that are Flu A+/B- and Flu A-/B+ are provided with the kit. Process swabs in accordance with Procedure Step 3 (a) for swab specimens without transport media. If controls do not perform as expected, do not report patient results.

**LIMITATIONS**

- Both viable and nonviable influenza A and B viruses are detectable with the Xpect™ Flu A&B test.
- Due to low levels of virus shedding, inadequate specimen collection or improper handling or transport, a negative test result does not rule out the presence of influenza virus. Consequently, the results from the Xpect™ Flu A&B test should be used in conjunction with other clinical findings to establish a diagnosis.
- A positive test does not rule out the possibility of co-infection with another pathogen.
- The performance characteristics of the Xpect™ Flu A&B test have not been established for use in monitoring antiviral treatment or for cell culture confirmation/identification methods.

**EXPECTED VALUES**

In the United States, influenza is most prevalent during the winter months. During peak periods, up to 30% of specimens tested may be culture positive for influenza. The proportion of influenza A positive specimens compared to influenza B can vary dramatically from year to year, ranging from about 50% to 99%.

**PERFORMANCE CHARACTERISTICS**

**Clinical Accuracy:**

The performance of the Xpect™ Flu A&B was evaluated at three sites located in the north, south, and east regions of the United States. The clinical trial sites included a Children's hospital (pediatric population), a University hospital (primarily adult population), and a reference laboratory (adult and pediatric (60/40) population). For all specimens evaluated, the overall sensitivity of the Xpect™ Flu A&B test when compared to culture was 92.2% (71/77) for influenza A and 97.8% (45/46) for influenza B. The overall specificity was 100% for both influenza A (314/314) and influenza B (345/345). For influenza A, there were 6 samples that were culture positive and Xpect™ Flu A&B negative. For influenza B, there was 1 sample that was culture positive and Xpect™ Flu A&B negative. Four of five discrepant samples available for analysis were positive by RT-PCR.

**Nasal Wash (n=239)**

Influenza A  
92.5% Sensitivity (37/40); 95% CI = 79.6-98.4%  
100% Specificity (199/199); 95% CI = 98.2-100%

Influenza B  
100% Sensitivity; (36/36); 95% CI = 90.3-100%  
100% Specificity (203/203); 95% CI = 98.2-100%

		Culture Results		
		A+ / B-	A- / B+	A- / B-
<b>Xpect™ Flu A&amp;B Results</b>	A+ / B-	37	0	0
	A- / B+	0	36	0
	A- / B-	3*	0	163

\*RT-PCR was performed on the three discrepant results. One of the three specimens was negative by PCR, two were positive.

Test performance by individual site:

FLU A		Sensitivity			Specificity		
Site	#	%	95% CI	#	%	95% CI	
1	0/0	NA	NA	1/1	100	NA	
2	3/5	60.0	14.7-94.7	69/69	100	94.8-100	
3	34/35	97.1	85.1-99.9	129/129	100	97.2-100	

FLU B		Sensitivity			Specificity		
Site	#	%	95% CI	#	%	95% CI	
1	0/0	NA	NA	1/1	100	NA	
2	0/0	NA	NA	74/74	100	95.1-100	
3	36/36	100	90.3-100	128/128	100	97.2-100	

**Throat Swab (n=30)**

Influenza A  
100% Sensitivity (10/10); 95% CI = 69.2-100%  
100% Specificity (20/20); 95% CI = 83.2-100%

Influenza B  
100% Sensitivity; (4/4); 95% CI = 39.8-100%  
100% Specificity (26/26); 95% CI = 86.8-100%

OVERALL		Culture Results		
		A+ / B-	A- / B+	A- / B-
<b>Xpect™ Flu A&amp;B Results</b>	A+ / B-	10	0	0
	A- / B+	0	4	0
	A- / B-	0	0	16

Test performance by individual site:

FLU A		Sensitivity			Specificity		
Site	#	%	95% CI	#	%	95% CI	
1	10/10	100	69.2-100	18/18	100	81.5-100	
2	0/0	NA	NA	2/2	100	15.8-100	
3	NA	NA	NA	NA	NA	NA	

FLU B		Sensitivity			Specificity		
Site	#	%	95% CI	#	%	95% CI	
1	4/4	100	39.8-100	24/24	100	85.8-100	
2	0/0	NA	NA	2/2	100	15.8-100	
3	NA	NA	NA	NA	NA	NA	

**Nasal Swab (n=122)**

Influenza A  
88.9% Sensitivity (24/27); 95% CI = 70.8-97.7%  
100% Specificity (95/95); 95% CI = 96.2-100%

Influenza B  
83.3% Sensitivity; (5/6); 95% CI = 35.9-99.6%  
100% Specificity (116/116); 95% CI = 96.9-100%

OVERALL		Culture Results		
		A+ / B-	A- / B+	A- / B-
<b>Xpect™ Flu A&amp;B Results</b>	A+ / B-	24	0	0
	A- / B+	0	5	0
	A- / B-	3*	1*	89

\*RT-PCR was performed on two of the four discrepant specimens that were available (one influenza A and one influenza B). Both specimens were positive by PCR.

Test performance by individual site:

FLU A		Sensitivity			Specificity		
Site	#	%	95% CI	#	%	95% CI	
1	24/27	88.9	70.8-97.7	91/91	100	96.0-100	
2	0/0	NA	NA	4/4	100	39.8-100	
3	NA	NA	NA	NA	NA	NA	

FLU B		Sensitivity			Specificity		
Site	#	%	95% CI	#	%	95% CI	
1	5/6	83.3	35.9-99.6	112/112	100	96.8-100	
2	0/0	NA	NA	4/4	100	39.8-100	
3	NA	NA	NA	NA	NA	NA	

**Analytical Sensitivity:**

The analytical sensitivity was evaluated using 12 influenza strains; 6 influenza A and 6 influenza B. Each viral strain was quantitated by CEID<sub>50</sub> determinations and titrated until a positive endpoint was reached using the Xpect™ Flu A&B test. The amount of virus at the endpoint dilution, expressed as CEID<sub>50</sub> per test, was calculated as a measure of analytical sensitivity.

Influenza Strain	Type	Detection Limit CEID <sub>50</sub>
A/Puerto Rico/8/34 (H1N1)	A	8.9 x 10 <sup>3</sup>
A/Fort Monmouth/1/47 (H1N1)	A	7.9 x 10 <sup>1</sup>
A/New Jersey/8/76 (H1N1)	A	8.9 x 10 <sup>1</sup>
A/Hong Kong/8/68 (H3N2)	A	2.8 x 10 <sup>1</sup>
A/Victoria/3/75 (H3N2)	A	8.9 x 10 <sup>2</sup>
A/Port Chalmers/1/73 (H3N2)	A	4.0 x 10 <sup>1</sup>
B/Lee/40	B	7.9 x 10 <sup>3</sup>
B/Allen/45	B	4
B/Maryland/1/59	B	6
B/GL/1739/54	B	8.9 x 10 <sup>1</sup>
B/Taiwan/2/62	B	3
B/Hong Kong/5/72	B	1.58 x 10 <sup>2</sup>

**Cross-Reactivity:**

Thirty-six microorganisms were evaluated with the Xpect™ Flu A&B test. No cross-reactivity was observed for influenza A or influenza B. Bacteria and yeast isolates were tested at 10<sup>6</sup> colony-forming units per ml concentration. Viral isolates were tested at concentrations of 10<sup>4</sup> to 10<sup>5</sup> TCID<sub>50</sub> (tissue culture infectious dose) per ml concentration. The following organisms were tested in the Xpect™ Flu A&B test.

<i>Acinetobacter baumannii</i>	<i>Serratia marcescens</i>
<i>Bordetella pertussis</i>	<i>Staphylococcus aureus</i> (Cowan)
<i>Candida albicans</i>	<i>Staphylococcus epidermidis</i>
<i>Enterococcus faecalis</i>	<i>Streptococcus mutans</i>
<i>Escherichia coli</i>	<i>Streptococcus pneumoniae</i>
<i>Gardnerella vaginalis</i>	<i>Streptococcus pyogenes</i> Group A
<i>Haemophilus influenzae</i>	<i>Streptococcus</i> , Group B
<i>Klebsiella pneumoniae</i>	<i>Streptococcus</i> , Group C
<i>Lactobacillus casei</i>	<i>Streptococcus</i> , Group F
<i>Legionella pneumophila</i>	Adenovirus, Type 5
<i>Listeria monocytogenes</i>	Coronavirus
<i>Moraxella catarrhalis</i>	Coxsackievirus B5
<i>Neisseria gonorrhoeae</i>	Cytomegalovirus
<i>Neisseria meningitidis</i>	Parainfluenza (Sendai), Type 1
<i>Neisseria sicca</i>	Parainfluenza, Type 2
<i>Neisseria subflava</i>	Parainfluenza, Type 3
<i>Proteus vulgaris</i>	Respiratory Syncytial Virus, A
<i>Pseudomonas aeruginosa</i>	Rhinovirus, Type 14

**Interfering Substances:**

The following substances were tested with the Xpect™ Flu A&B test and no interference was observed in the assay for any substance tested at the indicated levels: whole blood (2%), 3 mouthwashes (25%), 3 throat drops (25%), 3 nasal sprays (25%), 4-acetamidophenol (acetaminophen) (10 mg/ml), acetylsalicylic acid (20 mg/ml), chlorpheniramine (5 mg/ml), dextromethorphan (10 mg/ml), diphenhydramine (5 mg/ml), guaicol glyceryl ether (guaifenesin) (20 mg/ml), oxymetazoline (10 mg/ml), phenylephrine (25 mg/ml), phenylpropanolamine (20 mg/ml).

**Reproducibility:**

Reproducibility testing was conducted at four sites, including one in-house site, on four separate days with six blinded samples. The liquid samples consisted of diluted influenza A and influenza B antigens intended to read weakly positive or negative with the Xpect™ Flu A&B test. Ninety-nine percent of the 96 samples tested produced the expected result.




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**PACKAGING**

REF 24600, Xpect™ Flu A&B ..... 20 Tests/Kit

**SYMBOL LEGEND**

<b>REF</b>	Catalog Number
<b>IVD</b>	In Vitro Diagnostic Medical Device
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
<b>LOT</b>	Batch Code (Lot Number)
	Use By (Expiration Date)

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12076 Santa Fe Drive, Lenexa, KS 66215, USA  
 General Information: (800) 255-6730 Technical Service: (800) 447-3641 Order Entry: (800) 447-3635  
 Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128  
 Website: www.remel.com Email: remel@remel.com