Rapid Response®

4-in-1 Flu A/B, RSV & Adenovirus Antigen Test Pen
(Nasal Secretions)

REF FRA-19P, FRA-19PPC1,
FRA-19PPC5, FRA-19PPC25

Product Insert

For laboratory *in vitro* diagnostic use only.

Intended Use

The Rapid Response® 4-in-1 Flu A/B, RSV & Adenovirus Antigen Test Pen is an *in vitro* immunoassay. The assay is for the direct and qualitative detection of antigens of adenovirus (ADV), influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) from nasal secretions. The test is for laboratory professional use and can detect one or more viral antigens.
Negative results do not preclude these infections and should be confirmed by PCR. Test results should not be used as a sole basis for diagnosis but recommended to interpreted by a physician in the clinical context.

Principle

The Rapid Response® 4-in-1 Flu A/B, RSV & Adenovirus Antigen Test Pen detects viral antigens through visual interpretation of colour development on the three internal test strips for ADV, FLU A/B and RSV respectively.

For Adenovirus test:

Anti-adenovirus antibodies are immobilized at the test region of the nitrocellulose membrane. anti-adenovirus antibodies conjugated to coloured particles are immobilized on the conjugated pad.
The nasal secretions, collected by the intended user, are supposed to be mixed with the extraction buffer, which is individually packed in the kit.
During testing, target antigens, if present in the nasal secretions, will be released into the extraction buffer. As the specimen migrates along the strip by capillary action and then interacts with reagents on the Sample Pad, the target antigens will bind to anti-adenovirus antibodies on the Conjugate Pad. Consequently, the antigen-antibody complex will be captured by the anti-adenovirus antibodies immobilized at the Test Region. Excess coloured particles will be captured at the Control Region of the NC membrane.
The presence of a coloured line in the test region indicates a positive result for the adenovirus antigens, while its absence indicates a negative result. A coloured line at the control region serves as a procedural control, generally indicating that a proper volume of specimen has been added and membrane wicking is working.

For Influenza A/B test:

Anti-Influenza A virus antibodies and anti-Influenza B virus antibodies are immobilized at two separate test regions of the nitrocellulose membrane. Anti-Influenza A virus antibodies and anti-Influenza B virus antibodies conjugated to coloured particles are immobilized on the conjugated pad.
The nasal secretions, collected by the intended user, are supposed to be mixed with the extraction buffer, which is individually packed in the kit.
During testing, target antigens, if present in the nasal secretions, will be released into the extraction buffer. As the specimen migrates along the strip by capillary action and then interacts with reagents on the Sample Pad, the target antigens will bind to antibodies on the Conjugate Pad. Consequently, the antigen-antibody complex will be captured by the antibodies immobilized at the two Test Regions. Excess coloured particles will be captured at the Control Region of the NC membrane.
The presence of a coloured line in the test region indicates a positive result for the Influenza A/B viral antigens, while its absence indicates a negative result. A coloured line at the control region serves as a procedural control, generally indicating that a proper volume of specimen has been added and membrane wicking is working.

For RSV test:

Anti-respiratory syncytial virus antibodies are immobilized at the test region of the nitrocellulose membrane. Anti-respiratory syncytial virus antibodies conjugated to coloured particles are immobilized on the conjugated pad.
The nasal secretions, collected by the intended user, are supposed to be mixed with the extraction buffer, which is individually packed in the kit.
During testing, target antigens, if present in the nasal secretions, will be released into the extraction buffer. As the specimen migrates along the strip by capillary action and then interacts with reagents on the Sample Pad, the target antigens will bind to anti-respiratory syncytial virus antibodies on the Conjugate Pad. Consequently, the antigen-antibody complex will be captured by the anti-respiratory syncytial virus antibodies immobilized at the Test Region. Excess coloured particles will be captured at the Control Region of the NC membrane.
The presence of a coloured line in the test region indicates a positive result for the respiratory syncytial viral antigens, while its absence indicates a negative result. A coloured line at the control region serves as a procedural control, generally indicating that a proper volume of specimen has been added and membrane wicking is working.

Precautions

- For laboratory *in vitro* diagnostic use only.
- Caution should be taken when inserting the sample collector into the nasal cavity.
- Do not ingest.
- Read the Package Insert prior to use. Directions should be read and followed carefully.
- Do not use kit or components beyond the expiration date.
- Test devices are packaged in foil pouches that exclude moisture during storage. Inspect each foil pouch before opening. Do not use devices that have holes in the foil or where the pouch has not been completely sealed. Erroneous result may occur if test reagents or components are improperly stored.
- All patient specimens should be handled and discarded as if they are biologically hazardous. All specimens must be mixed thoroughly before testing to ensure a representative sample prior to testing.
- Failure to bring specimens and reagents to room temperature before testing may decrease assay sensitivity. Inaccurate or inappropriate specimen collection, storage, and transport may yield false negative test results.
- Avoid skin or eyes contact with buffer before, during or after testing.
- Do not puncture the sealing membrane in the extraction tube before testing.

Materials

Materials provided

- Individually packed test Pen
- Desiccant
- Product insert

Materials required but not provided

- Clock, timer or stopwatch

Storage and Stability

- Store the test at 2~30°C (35.6~86°F) when not in use.
- **DO NOT FREEZE.**
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.

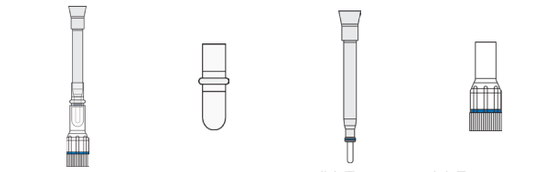
Test Procedure

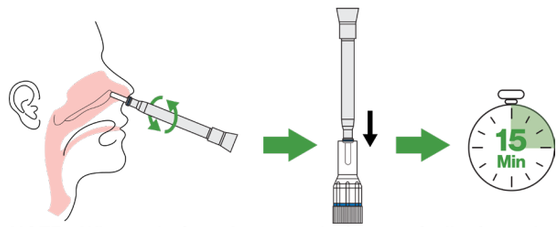
Bring devices, reagents and specimens and/or controls to room temperature (15~30°C; 59~86°F) before use.

1. Remove the test from its packing. Label the device with the patient's identification. For best results, the assay should be performed within one hour.
2. 1) Take the test device out of the extraction tube.
   2) Remove the protector.
3. Gently insert the sample collector (the swab tip of the pen) until resistance is met (about 1-2 cm into the nostril).
4. Rotate the collector **five times** against the nasal wall and remove from the nostril.
5. **Repeat the sample collection procedure for the other nostril** to ensure that sufficient specimen be collected from both nasal cavities.

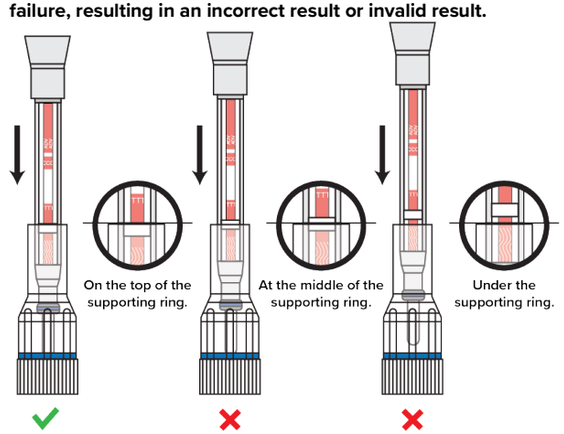
NOTE: 1. It is important to obtain as much secretion as possible.
2. This may feel uncomfortable. Do not insert the collector any deeper if patients feel strong resistance.

6. Place the test device vertically into the extraction tube until the top edge of the extraction tube reach the top of the supporting ring.
7. Read the results at 15 minutes.



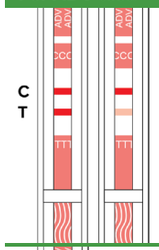


NOTE: When placing the test device vertically into the extraction tube, the edge of the extraction tube must reach the top of the supporting ring. If not, this may lead to lateral flow failure, resulting in an incorrect result or invalid result.

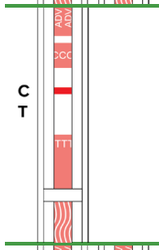


Results Interpretation

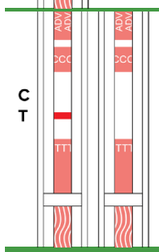
For ADV test:



POSITIVE: Two coloured lines appear on the membrane. One line appears in the control region (C) and another line appears in the test region (T).

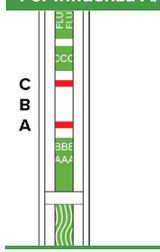


NEGATIVE: Only one coloured line appears, in the control region (C). No apparent coloured line appears in the test region (T).

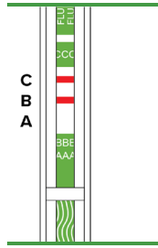


INVALID: Control line fails to appear. Results from any test which has not produced a control line at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

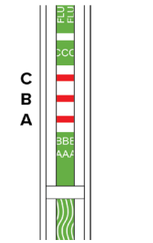
For Influenza A/B test:



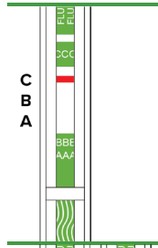
Influenza A Positive: One coloured line appears in the control region (C), and another coloured line in the A region (A).



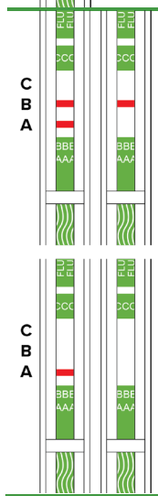
Influenza B Positive: One coloured line appears in the control region (C), and another coloured line in the B region (B).



Influenza A+B Positive: One coloured line appears in the control region (C), and a coloured lines appear in both A region (A) and B region (B).
NOTE: Co-infection with influenza A and B is rare. A clinical specimen that generates positive results for both A and B should be considered an invalid result, and another test should be performed. If the test is again positive for both influenza A and B, the specimen should be re-tested by another method prior to reporting of results.

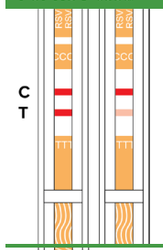


Negative: Only one coloured line appears in the control region (C), and no line appears neither in the A region (A) nor B region (B).

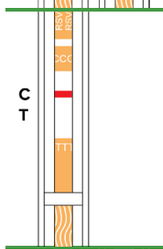


Invalid: No coloured line appears in the control region (C), whether a test line(s) is present or not. Repeat invalid tests with a new sample, new test device and reagent. Insufficient sample volume, inaccurate operating procedure or expired tests may yield an invalid result. Contact your local distributor if the problem continues.

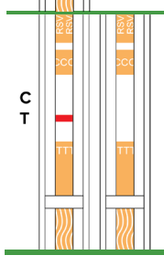
For RSV test:



POSITIVE: Two coloured lines appear on the membrane. One line appears in the control region (C) and another line appears in the test region (T).



NEGATIVE: Only one coloured line appears, in the control region (C). No apparent coloured line appears in the test region (T).



INVALID: Control line fails to appear. Results from any test which has not produced a control line at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

NOTE:

1. The colour intensity in the test region(s) may vary depending on the concentration of analytes present in the specimen. Note that this is a qualitative test only, and cannot determine the concentration of analytes in the specimen.

Quality Control

Internal Procedural Controls

The Rapid Response® 4-in-1 Flu A/B, RSV & Adenovirus Antigen Test Pen has built-in (procedural) controls. Each test has an internal standard zone to ensure proper sample flow. The user should confirm that the coloured line located at the “C” region is present before reading the result.

External Positive and Negative Controls

Good laboratory practice suggests testing positive and negative external controls to ensure that the test reagents are working and that the test is correctly performed.

Limitations

1. The Rapid Response® 4-in-1 Flu A/B, RSV & Adenovirus Antigen Test Pen is for professional *in vitro* diagnostic use, and should only be used for the qualitative detection of antigens specific for adenovirus, Influenza A virus, Influenza B virus and respiratory syncytial virus. The intensity of colour in a positive line should not be evaluated as “quantitative or semi-quantitative”.
2. Both viable and nonviable viruses are detectable with the kit.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
4. Failure to follow the TEST PROCEDURE and RESULT INTERPRETATION may adversely affect test performance and/or invalidate the test result.
5. Results obtained with this assay, particularly in the case of weak test lines that are difficult to interpret, should be used in conjunction with other clinical information available to the physician.
6. Negative results do not preclude viral infections and should be confirmed via molecular assay.

Performance Characteristics

Analytical Sensitivity

Limit of detection (LOD) for Adenovirus and influenza A/B and RSV in the Rapid Response® 4-in-1 Flu A/B, RSV & Adenovirus Antigen Test Pen was determined by evaluating different concentrations of inactivated viruses. Natural nasal swab specimens were obtained from healthy donors and confirmed negative for Adenovirus and influenza A/B and RSV. Negative natural nasal swab specimens were eluted in PBS. Swab elutes were combined and mixed thoroughly to create a negative clinical matrix pool to be used as the diluent. The viruses were diluted in this natural nasal swab matrix pool to generate virus dilutions for testing. Nasal swab samples were prepared by adding 50µL of each virus dilution onto the sterile swab.

The swab samples were tested according to the test procedure in the product insert. The LOD listed below:

Virus Strains	Limit of detection (LoD)
Influenza A (H1N1)	1.0×10^4 $TCID_{50}$/mL
Influenza A (H3N2)	4.3×10^4 $TCID_{50}$/mL
Influenza B (Yamagata)	2.5×10^5 $TCID_{50}$/mL
Influenza B (Victoria)	2.2×10^5 $TCID_{50}$/mL
Respiratory syncytial virus, type A	9.0×10^3 $TCID_{50}$/mL
Respiratory syncytial virus, type B	2.4×10^3 $TCID_{50}$/mL
Adenovirusm (Type 4)	1.4×10^3 $TCID_{50}$/mL

Clinical Evaluation

For Adenovirus (ADV) test:

A total of 652 clinical specimens were collected, to verify the performance of Adenovirus Antigen Test. 37 were found to be positive by RT-PCR and 615 were found to be negative by RT-PCR.

Table 1: Clinical Summary of ADV

		RT-PCR		Total
		Positive	Negative	
Adenovirus Test	Positive	34	1	35
	Negative	3	614	617
	Total	37	615	652

Relative Sensitivity:91.9% (78.7%-97.2%)*
Relative Specificity: 99.8% (99.1%-100.0%)*
Overall Agreement: 99.4% (98.4%-99.8%)
*95% Confidence Interval

For FLU A/B Test:

For Influenza A Test:

A total of 652 clinical specimens were collected, to verify the performance of Influenza A/B Antigen Test. 70 were found to be positive by RT-PCR and 582 were found to be negative by RT-PCR.

For Influenza B Test:

A total of 652 clinical specimens were collected, to verify the performance of Influenza A/B Antigen Test. 33 were found to be positive by RT-PCR and 619 were found to be negative by RT-PCR.

Table 2: Clinical Summary of Influenza A

		RT-PCR		Total
		Positive	Negative	
Influenza A Test	Positive	66	6	72
	Negative	4	576	580
	Total	70	582	652

Diagnostic Sensitivity: 94.3% (86.2% ~97.8%)*
Diagnostic Specificity: 99.0% (97.8% ~99.5%)*
Overall Agreement: 98.5% (97.2% ~ 99.2%)*
*95% Confidence Interval

Table 3: Clinical Summary of Influenza B

		RT-PCR		Total
		Positive	Negative	
Influenza B Test	Positive	31	5	36
	Negative	2	614	616
	Total	33	619	652

Diagnostic Sensitivity: 93.9% (80.4% ~98.3%)*
Diagnostic Specificity: 99.2% (98.1% ~99.7%)*
Overall Agreement: 98.9% (97.8% ~ 99.5%)*
*95% Confidence Interval

For Respiratory Syncytial Virus (RSV) test:

A total of 652 clinical specimens were collected, to verify the performance of Respiratory Syncytial Virus Antigen Test. 35 were found to be positive by RT-PCR and 617 were found to be negative by RT-PCR.

Table 4: Clinical Summary of RSV

		RT-PCR		Total
		Positive	Negative	
Respiratory Syncytial Virus Test	Positive	32	6	38
	Negative	3	611	614
	Total	35	617	652

Diagnostic Sensitivity: 91.4% (77.6% ~97.0%)*
Diagnostic Specificity: 99.0% (97.9% ~99.6%)*
Overall Agreement: 98.6% (97.4% ~ 99.3%)*
*95% Confidence Interval

Cross Reactivity

Cross reactivity with the following organisms has been studied. Samples positive for the following organisms were found negative when tested with the Rapid Response® 4-in-1 Flu A/B, RSV & Adenovirus Antigen Test Pen.

Epstein-Barr virus	Enterovirus EV70
Enterovirus EV71	Enterovirus A16
Enterovirus A24	Enterovirus B1
Echovirus 6	HCoV-229E
HCoV-OC43	HCoV-NL63
MERS-coronavirus	Human metapneumovirus
Norovirus	Parainfluenza virus
Respiratory syncytial virus B	Respiratory syncytial virus A
Rhinovirus B52	Rhinovirus A30
Bordetella pertussis	Bordetella parapertussis
Chlamydia pneumoniae	Candida albicans
Haemophilus influenzae	Group C Streptococcus
Mycoplasma pneumoniae	Legionella pneumophila
Staphylococcus aureus	Mycobacterium tuberculosis
Streptococcus agalactiae	Staphylococcus epidermidis
Streptococcus pyogenes	Streptococcus pneumoniae

NOTE:

1. **For ADV test:** Adenovirus detection has no cross reactivity with influenza A, influenza B, and respiratory syncytial virus.
2. **For FLU A/B test:** FLU A detection has no cross reactivity with influenza B, respiratory syncytial virus, and adenovirus. FLU B detection has no cross reactivity with influenza A, respiratory syncytial virus, and adenovirus.

Interfering Substances

The following substances, naturally present in respiratory specimens or that may be artificially introduced into the respiratory tract, were evaluated at the concentrations listed below. None of them were found to affect test performance of the kit.

Substance	Concentration
3 OTC nasal sprays	10%
3 OTC mouth washes	10%
3 OTC throat drops	10%
4-acetamidophenol	10 mg/mL
Acetylsalicylic acid	10 mg/mL
Albuterol	10 mg/mL
Chlorpheniramine	5 mg/mL
Dexamethasone	50 µg/mL
Dextromethorphan	10 µg/mL
Diphenhydramine	5 mg/mL
Doxylamine succinate	1 mg/mL
Flunisolide	25 µg/mL
Guaiacol glyceryl ether	20 mg/mL
Mucin	1%
Whole blood	4%
Mupirocin	250 µg/mL
Oxymetazoline	25 µg/mL
Phenylephrine	10 mg/mL
Phenylpropanolamine	1 mg/mL
Zanamivir	10 mg/mL
Adamantanamine	500 ng/mL
Oseltamivir phosphate	10 mg/mL
Tobramycin	10 mg/mL
Triamcinolone	14 mg/mL

Bibliography

1. Voss, Jameson D.; Atkinson, Richard L.; Dhurandhar, Nikhil V. (1 November 2015). "Role of adenoviruses in obesity". Rev. Med. Virol. 25(6): 379–387. doi:10.1002/rmv.1852. PMID 26352001.
2. Wadell G.; et al. (1987). Whelan, Julie; Bock, Gregory (eds.). Novel diarrhoea viruses. New York: Wiley. p. 63. ISBN 978-0-471-91094-7.
3. Wikipedia contributors, "Influenza virus C," Wikipedia, The Free Encyclopedia, http://en.wikipedia.org/w/index.php?title=Influenzavirus_C&oldid=649896527 (accessed May 25, 2015).
4. Course BS3035: Virology, University of Leicester, http://www-micro.msb.le.ac.uk/3035/Paramyxoviruses.html.
5. Respiratory Syncytial Virus (RSV): Overview, Treatment, and Prevention Strategies, Mark J. Polak, MD.
6. Macartney K. et al. Nosocomial Respiratory Syncytial Virus Infections: The Cost-Effectiveness and Cost-Benefit of Infection Control. Pediatrics Vol. 106 No. 3 Sept 2000, pp. 520–526. http://pediatrics.aappublications.org/cgi/content/full/106/3/520.
7. Thompson W. et al. Mortality Associated With Influenza and Respiratory Syncytial Virus in the United States. JAMA, January 8, 2003 – Vol 289, No. 2.
8. Moler F.W. et al. Respiratory syncytial virus morbidity and mortality estimates in congenital heart disease patients: a recent experience. Crit Care Med. 1992 Oct; 20(10):1406–13.

Glossary of Symbols

Consult instructions for use
Test per Kit
Do Not Reuse
Store between 2~30°C (35.6~86°F)
Use by
REF Catalogue #
LOT Lot Number
IVD For *in vitro* diagnostic use only
Manufacturer

BTNX Inc.
722 Rosebank Road,
Pickering, ON L1W 4B2
Canada
Technical Support: 1-888-339-9964