Rapid Response®

Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen

(Nasal Secretions)

REF COF-19PPC2 Product Insert
COF-19PPC5
COF-19PPC10
COF-19PPC20

For point-of-care use only.

Intended Use

The Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen is *an in vitro* immunoassay. The assay is for the direct and qualitative detection of viral nucleocapsid proteins of SARS-CoV-2, Influenza A virus, and Influenza B virus from nasal secretions from individuals suspected of Influenza A, B or COVID-19 infection within the first 7 days of symptoms onset. This test is intended for point of care use only. It is not intended for home testing (or self-testing).

Results are for identification of Influenza A and B and SARS-CoV-2 viral nucleoprotein antigen. Antigen is generally detectable in nasal secretions during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories are required to report all positive results to the appropriate public health authority.

Negative results do not rule out Influenza A and B and SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with Influenza A and B and COVID-19, and confirmed with a molecular assay, if necessary for patient management.

People who are suspected of being infected with SARS-CoV-2 virus:

Negative SARS-CoV-2 results should be treated as presumptive. To increase the chance that the negative result is accurate, test again 48 hours after the first negative result

The assay is for point of care use only, and the intended user is a healthcare professional.

Principle

The Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen detects viral antigens through visual interpretation of color development on the two internal test strips for COVID-19 and FLU A/B respectively.

For COVID-19 test:

Anti-SARS-CoV-2 antibodies are immobilized at the test region of the nitrocellulose membrane. Anti-SARS-CoV-2 antibodies conjugated to colored particles are immobilized on the conjugated pad.

The nasal secretions, collected by the intended user, is supposed to be mixed with the extraction buffer, which is individually packed in the kit.

During testing, antigens which are present in the nasal secretions will be released into the extraction buffer, the target antigens will bind to anti-SARS-CoV-2 antibodies conjugated to colored particles. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by the anti-SARS-CoV-2 antibodies in the test region. Excess colored particles are captured in the internal control zone.

The presence of a colored band in the test region indicates a positive result for the SARS-CoV-2 viral antigens, while its absence indicates a negative result. A colored band 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 colored particles are immobilized on the conjugated pad.

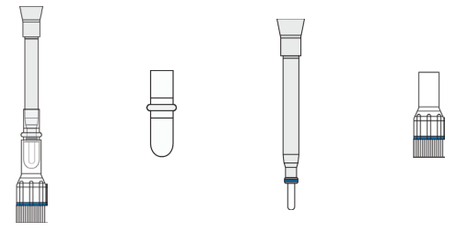
The nasal secretions, collected by the intended user, is supposed to be mixed with the extraction buffer, which is individually packed in the kit.

During testing, antigens which are present in the nasal secretions will be released into the extraction buffer, the target antigens will bind to anti-influenza A or/and B antibodies conjugated to colored particles. As the specimen migrates along the strip by capillary action and interacts with reagents on the membrane, the complex will be captured by antibodies in the respective detection zone. Excess colored particles are captured in the internal control zone.

The presence of a colored band in the test region indicates a positive result for the Influenza A/B viral antigens, while its absence indicates a negative result. A colored band 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 professional *in vitro* diagnostic use only.
- Caution should be taken when inserting the sample collector into the nasal cavity.



(a) Test (b) Protector (c) Pen (c) Cap (Extraction tube)

- Do not use the kit on children under 2 years old.
- 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 kits 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 results 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 test kits 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.
- If infections with SARS-CoV-2, Influenza A virus and/or Influenza B virus are suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions and sent to state or local health departments for testing.
- Do not puncture the sealing membrane in the Cap before testing.
- Viral isolation in cell culture and initial characterization of viral agents recovered in cultures of SARS-CoV-2, Influenza A virus, or Influenza B virus are NOT recommended, except in a BSL3 laboratory using BSL3 work practices.

Materials

Materials provided

- Individually packed test
- Package insert

Materials required but not provided

- Clock, timer or stopwatch

Storage and Stability

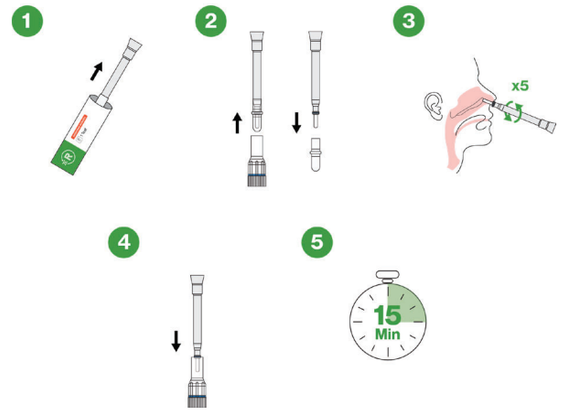
- Store the Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen at 2~30°C when not in use.
- **DO NOT FREEZE.**
- Kit contents are stable until the expiration dates marked on their outer packaging and containers.

Test Procedure

Bring test kits, specimens and/or controls to room temperature (15~30°C) before use.

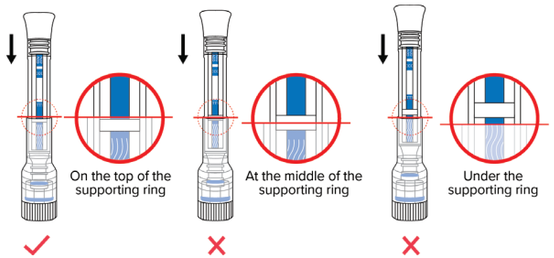
1. Remove the test from its packaging. Label the device with the patient's identification. For the best results, the assay should be performed within one hour. Take the pen out of the cap and remove the protector.
2. Gently insert the sample collector into the nostril until resistance is met (about 1-2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

   NOTE: Swabbing may feel uncomfortable, but it should not be painful. It is important to obtain as much secretion as possible. Do not insert the collection tip any deeper if strong resistance is felt.
4. Place the pen vertically into the cap until the top edge of the cap reaches the top of the supporting ring. See the illustration below for details.
5. Read the results at 15 minutes. Do not read the results after 30 minutes.



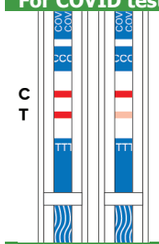
Note: When placing the pen vertically into the Cap, the edge of the Cap

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.

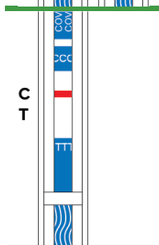


Results Interpretation

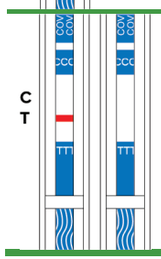
For COVID test:



POSITIVE: Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).

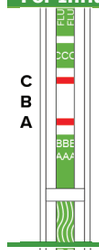


NEGATIVE: One colored band appears in the control region (C). No band appears in the test region (T).

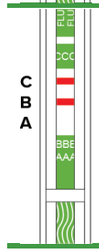


INVALID: Control band fails to appear. Results from any test which has not produced a control band at the specified read time must be discarded. Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

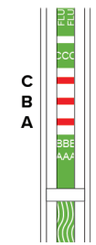
For Influenza A/B test:



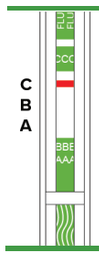
Influenza A Positive: One colored band appears in the control region (C), and another colored band in the A region (A).



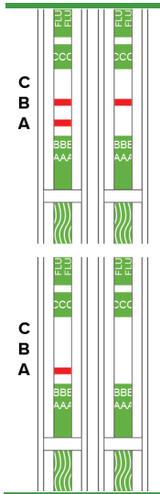
Influenza B Positive: One colored band appears in the control region (C), and another colored band in the B region (B).



Influenza A+B Positive: One colored band appears in the control region (C), and two other colored bands 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 colored band appears in the control region (C), and no band appears neither in the A region (A) nor B region (B).



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

Quality Control

Internal Procedural Controls

The Rapid Response® COVID-19 & Influenza A/B 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 colored band 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. External positive and negative controls should be used in accordance with applicable accrediting organizations.

Limitations

1. The Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen is for point of care in *vitro* diagnostic use, and should only be used for the qualitative detection of viral antigens specific for SARS-CoV-2, Influenza A virus, and Influenza B virus. The intensity of color in a positive band 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:

The limit of detection (LOD) of Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen was identified by evaluating different concentrations of inactivated SARS-CoV-2, inactivated influenza A virus (H3N2, H1N1) and inactivated influenza B virus (Victoria, Yamagata). The concentrations identified as the LOD levels for each strain tested are listed below.

Strain	Source	Limit of Detection (LoD)
SARS-CoV-2	hCoV-19/China/ZJ-NB841/2020	$1.0\times10^{2.4}$ $TCID_{50}$/mL
Influenza A (H1N1) virus	A/China/ZJ-HZ166/2018	4.3×10^{4} $TCID_{50}$/mL
Influenza A (H3N2) virus	A/China/ZJ-TZ314/2016	1.0×10^{4} $TCID_{50}$/mL
Influenza B Yamagata lineage virus	BY/China/ZJ-HZ415/2018	2.5×10^{5} $TCID_{50}$/mL
Influenza B Victoria lineage virus	BV/China/ZJ-HZ809/2019	2.2×10^{5} $TCID_{50}$/mL

Hook-effect

The highest concentration of inactivated SARS CoV 2 stock available ($1\times10^{6.4}$ $TCID_{50}$/mL), Influenza A (H1N1) (2.0×10^{6} $TCID_{50}$/mL), Influenza A (H3N2) (8.6×10^{6} $TCID_{50}$/mL), Influenza B Victoria lineage (4.4×10^{6} $TCID_{50}$/mL) and Influenza B Yamagata lineage (5.0×10^{6} $TCID_{50}$/mL) were tested. No hook effect occurred on the device.

Clinical Evaluation:

Total 559 clinical samples were enrolled in the study. For COVID-19, 119 positive samples and 440 negative samples, for Influenza A, 60 positive samples and 499 negative samples, for Influenza B, 37 positive samples and 522 negative samples. These samples were tested with both PCR and Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen. The results of all the nasal swab clinical data are summarized in following tables:

Table 1: COVID-19 Antigen Test vs. RT-PCR

		RT-PCR		Total
		Positive	Negative	
Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen	Positive	103	1	104
	Negative	16	439	455
	Total	119	440	559

Diagnostic Sensitivity: 86.6% (79.3% ~91.6%)*
Diagnostic Specificity: 99.8% (98.7% ~ 100.0%)*
Overall Agreement: 97.0% (95.2% ~ 98.1%)*
*95% Confidence Interval

Table 2: Influenza A Antigen Test vs. RT-PCR

		RT-PCR		Total
		Positive	Negative	
Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen	Positive	55	3	58
	Negative	5	496	501
	Total	60	499	559

Diagnostic Sensitivity: 91.7% (81.9% ~96.4%)*
Diagnostic Specificity: 99.4% (98.2% ~ 99.8%)*
Overall Agreement: 98.6% (97.2% ~ 99.3%)*
*95% Confidence Interval

Table 3: Influenza B Antigen Test vs. RT-PCR

		RT-PCR		Total
		Positive	Negative	
Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen	Positive	33	2	35
	Negative	4	520	524
	Total	37	522	559

Diagnostic Sensitivity: 89.2% (75.3% ~95.7%)*
Diagnostic Specificity: 99.6% (98.6% ~ 99.9%)*
Overall Agreement: 98.9% (97.7% ~ 99.5%)*
*95% Confidence Interval

Cross Reactivity:

Cross reactivity with the following organisms has been studied. Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen presented no cross-reactivity with these microorganisms below.

Human coronavirus 229E	Parainfluenza virus 4	*Candida albicans*
Human coronavirus OC43	Influenza A (H1N1)	*Bordetella pertussis*
Human coronavirus NL63	Influenza A (H3N2)	*Mycoplasma pneumoniae*
SARS-coronavirus-2	Influenza B virus victoria lineage	*Chlamydia pneumoniae*
Human coronavirus HKU1	Influenza B virus Yamagata lineage	*Legionella pneumophila*
Adenovirus	Enterovirus	*Staphylococcus aureus*
Human Metapneumovirus	Rhinovirus	*Staphylococcus epidermidis*
Parainfluenza virus 1	*Haemophilus influenzae*	Pooled human nasal wash
Parainfluenza virus 2	*Streptococcus pneumoniae*	SARS-CoV
Parainfluenza virus 3	*Streptococcus pyogenes*	MERS-CoV
Adenovirus 7	Epstein Barr Virus	Measles
Mumps	Respiratory syncytial virus Type A	Respiratory syncytial virus Type B
Rhinovirus A30	*Streptococcus salivarius*	*Corynebacterium diphtheriae*
Escherichia coli	*Lactobacillus casei*	*Moraxella catarrhalis*
Mycobacterium tuberculosis	*Neisseria meningitidis*	*Neisseria gonorrhoeae*
Pseudomonas aeruginosa	*Streptococcus pyogenes*	/

To estimate the likelihood of cross-reactivity with SARS-COV-2 of organisms that were not available for wet testing, *in silico* analysis using the basic local alignment search tool (BLAST) managed by the National Center for Biotechnology Information(NCBI) was used the degree of protein sequence homology.
For *Pneumocystis jirovecii (PJP)*, blast results showed none homology exists between the SRAS-COV-2 nucleocapsid protein and *Pneumocystis jirovecii (PJP)*. For Mycobacterium tuberculosis, blast results showed no homology exists between the SRAS-COV-2 nucleocapsid protein and *Mycobacterium tuberculosis.*

NOTE:

1. **For FLU A detection:** FLUA detection has no cross-reactivity with influenza B and SARS-CoV-2.
2. **For FLU B detection:** FLUB detection has no cross-reactivity with influenza A and SARS-CoV-2.
3. **For SARS-CoV-2 detection (COVID-19):** SARS-CoV-2 detection has no cross-reactivity with influenza A and influenza B.
4. A cross-reactivity with SARS was observed for the detection of SARS-CoV-2, but not for the detection of Flu A and Flu B.

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 Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen.

Substance	Concen-tration	Substance	Concen-tration
Whole Blood	4%	Fluticasone Propionate	5% v/v
Mucin	0.50%	Oseltamivir Phosphate	5 mg/mL
Chloraseptic (Benzocaine/ Menthol)	1.5 mg/mL	Zicam® COLD REMEDY	5% v/v
Naso GEL (NeilMed)	5% v/v	Sore Throat Phenol Spray	15% v/v
Phenylephrine	15% v/v	Homeopathic (Alkalol)	1:10 dilution
Oxymetazoline	15% v/v	Body&Hand lotion (Cerave)	0.5% (w/v)
Cromolyn	15% v/v	Hand Sanitizer with Aloe, 62% ethyl alcohol	0.5% (w/v)
Tobramycin	4 µg/mL	Hand Lotion (Eucerin)	0.5% (w/v)
Mupirocin	10 mg/mL	Hand soap liquid gel (soft soap)	10% (w/v)

NOTE:
For FluMist® Quadrivalent Influenza Intranasal Vaccine

Dilu-tion from stock	Concen-tration (V:V)	Panel member	Result		
			COV	FLUA	FLUB
1:64	1.60%	Negative control	15/15-	15/15+	15/15+
		COV low positive control	15/15+	15/15+	15/15+
		FLU A (H1N1) low positive control	15/15-	15/15+	15/15+
		FLU A (H3N2) low positive control	15/15-	15/15+	15/15+
		FLUB (Yamagata) low positive control	15/15-	15/15+	15/15+
		FLUB (Victoria) low positive control	15/15-	15/15+	15/15+
1:128	0.80%	Negative control	15/15-	15/15-	15/15-
		COV low positive control	15/15+	15/15-	15/15-
		FLU A (H1N1) low positive control	15/15-	15/15+	15/15-
		FLU A (H3N2) low positive control	15/15-	15/15+	15/15-
		FLUB (Yamagata) low positive control	15/15-	15/15-	15/15+
		FLUB (Victoria) low positive control	15/15-	15/15-	15/15+

Analytical Inclusivity Study

The limit of detection (LoD) of Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen was identified by evaluating different concentrations of different SARS-CoV-2, Flu A and Flu B strains. The concentrations identified as the LOD levels for each strain tested are listed below.

SARS-CoV-2	Viral strain	LoD
SARS-CoV-2	Wild type	$1.0\times10^{2.4}$ $TCID_{50}$/mL
	B.1.1.7 (Alpha)	$1.0\times10^{2.5}$ $TCID_{50}$/mL
	B.1.351 (Beta)	$1.8\times10^{2.2}$ $TCID_{50}$/mL
	B.1.617.2 (Delta)	$5\times10^{1.5}$ $TCID_{50}$/mL
	B.1.1.529.1 (Omicron BA.1)	$1.0\times10^{2.25}$ $TCID_{50}$/mL
	B.1.1.529.2 (Omicron BA.2)	1.0×10^{2} $TCID_{50}$/mL
Influenza A (H1N1)	A/Michigan/45/2015	1.0×10^{4} $TCID_{50}$/mL
	A/California/07/2009	1.65×10^{4} $TCID_{50}$/mL
	A/Brisbane/02/2018	1.2×10^{4} $TCID_{50}$/mL
	A/Victoria/2570/2019	9.0×10^{3} $TCID_{50}$/mL
	A/Wisconsin/588/2019	1.48×10^{4} $TCID_{50}$/mL
	A/Sydney/5/2021	1.4×10^{4} $TCID_{50}$/mL
Influenza A (H3N2)	A/Singapore/INFIMH-16-0019/2016	1.78×10^{4} $TCID_{50}$/mL
	A/Hong Kong/4801/2014	4.3×10^{4} $TCID_{50}$/mL
	A/Hong Kong/2671/2019	9.8×10^{3} $TCID_{50}$/mL
	A/Hong Kong/45/2019	1.73×10^{4} $TCID_{50}$/mL
	A/Switzerland/9715293/2013	1.63×10^{4} $TCID_{50}$/mL
	A/Darwin/6/2021	8.4×10^{3} $TCID_{50}$/mL
	A/Darwin/9/2021	7.3×10^{3} $TCID_{50}$/mL
Influenza B (Yamagata lineage)	B/Massachusetts/2/2012	7.63×10^{4} $TCID_{50}$/mL
	B/Phuket/3073/2013	2.5×10^{5} $TCID_{50}$/mL
Influenza B (Victoria lineage)	B/Colorado/06/2017	2.2×10^{5} $TCID_{50}$/mL
	B/Brisbane/60/2008	5.5×10^{4} $TCID_{50}$/mL
	B/Washington/02/2019	8×10^{4} $TCID_{50}$/mL
	B/Austria/1359417/2021	5.5×10^{4} $TCID_{50}$/mL

Precision Study

The precision of Rapid Response® COVID-19 & Influenza A/B Antigen Test Pen was identified by Repeatability and reproducibility. Repeatability assessed intra-assay variability between replicates of panel members tested with the same lot, operator, site and day; reproducibility assessed the inter-assay variability between lots, operators, sites and days. The final data analysis is listed below.

Table 1: Data Analysis of the Intra-Assay Results

Panel member	Test Item			Overall agreement
	COV	FLUA	FLUB	
Negative control	80/80 -	80/80 -	80/80 -	240/240, 100%
COV low positive control	80/80 +	80/80 -	80/80 -	240/240, 100%
COV high positive control	80/80 +	80/80 -	80/80 -	240/240, 100%
FLUA low positive control	80/80 -	80/80 +	80/80 -	240/240, 100%
FLUA high positive control	80/80 -	80/80 +	80/80 -	240/240, 100%
FLUB low positive control	80/80 -	80/80 -	80/80 +	240/240, 100%
FLUB high positive control	80/80 -	80/80 -	80/80 +	240/240, 100%

Data Analysis of the Inter-Assay Results

Panel member	Test Item			Overall agreement
	COV	FLUA	FLUB	
Negative control	675/675 -	675/675 -	675/675 -	2025/2025, 100%
COV low positive control	675/675 +	675/675 -	675/675 -	2025/2025, 100%
COV high positive control	675/675 +	675/675 -	675/675 -	2025/2025, 100%
FLUA low positive control	675/675 -	675/675 +	675/675 -	2025/2025, 100%
FLUA high positive control	675/675 -	675/675 +	675/675 -	2025/2025, 100%
FLUB low positive control	675/675 -	675/675 -	675/675 +	2025/2025, 100%
FLUB high positive control	675/675 -	675/675 -	675/675 +	2025/2025, 100%

Hook Effect

The highest concentration of inactivated SARS CoV 2 stock available ($1\times10^{6.4}$ $TCID_{50}$/mL), Influenza A (H1N1) (2.0×10^{6} $TCID_{50}$/mL), Influenza A (H3N2) (8.6×10^{6} $TCID_{50}$/mL), Influenza B Victoria lineage (4.4×10^{6} $TCID_{50}$/mL) and Influenza B Yamagata lineage (5.0×10^{6} $TCID_{50}$/mL) were tested. No hook effect occurred on the device.

Bibliography

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Glossary of Symbols

Consult instructions for use
Tests per Kit
UDI Unique Device Information
Store between 35.6°F to 86°F
Use by
Do Not Reuse
LOT Lot Number
IVD For *in vitro* diagnostic use only
REF Catalogue #
Do not use if product is damaged
Manufacturer

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