



**savyonDIAGNOSTICS**

*member of the gamida diagnostics division*

## savvy<sup>gen</sup> Flu A, Flu B & RSV

**Catalog number: 612-01HU / 612-01LU**

**Test kit for 48 determinations**

**Store at 2<sup>o</sup>-37<sup>o</sup>C (35.6<sup>o</sup>-98.6<sup>o</sup>F)**

**For Research Use Only**

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## **Intended Use**

The Savvygen™ Flu A, Flu B & RSV test allows the qualitative detection of Influenza A (Flu A), Influenza B (Flu B) and/or Human Respiratory Syncytial Virus (RSV) by real time RT-PCR in respiratory samples from symptomatic patients. The product is intended for use in the diagnosis of Flu A, Flu B and/or RSV alongside clinical data of the patient and other laboratory tests outcomes.

**For Research Use Only.**

## **Background**

Influenza (Flu), is a highly contagious infectious disease caused by influenza virus types A B and C. Influenza viruses are RNA viruses belonging to the family of *Orthomyxoviridae*. The most common symptoms include high fever, runny nose, sore throat, muscle pain, headache, and fatigue. These symptoms typically begin two days after exposure to the virus and last less than a week afterwards. The virus is spread by direct contact and indirect transmission.<sup>(1, 2)</sup> Complications of influenza may include viral pneumonia, secondary bacterial pneumonia, sinus infections and deterioration of previous health problems such as asthma or heart failure.<sup>(3)</sup>

Influenza types A and B cause annual outbreaks resulting in epidemics whereas Influenza type C cause only a milder disease. About three to five million cases worldwide of severe illness and about 250,000 to 500,000 deaths per year are due to this disease. The majority of influenza cases are caused by influenza types A and B.<sup>(4,5)</sup>

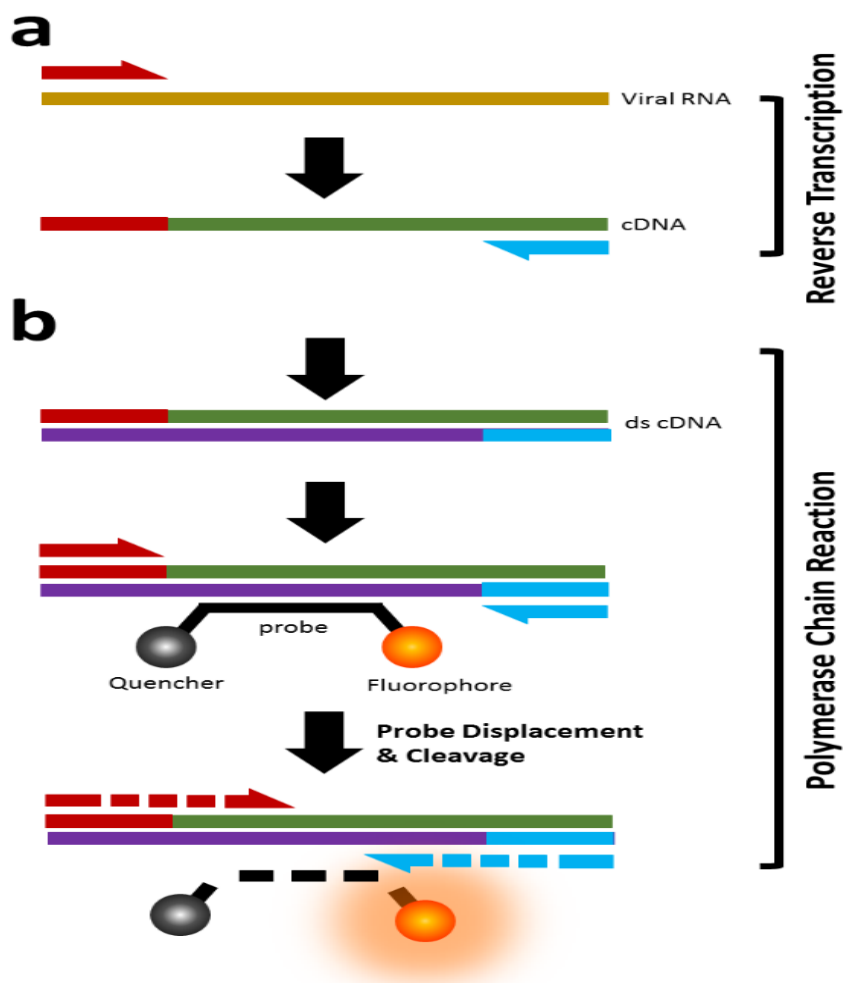
Human Respiratory Syncytial Virus (RSV) is a major cause of lower respiratory tract infections during infancy and childhood with 2 million outpatient visits of children younger than 5 years old in the USA alone.<sup>(6)</sup> In adults, RSV mainly produces mild symptoms, often indistinguishable from common colds, however in immunocompromised or prematurely born infants RSV can lead to more severe respiratory illness requiring hospitalization and, rarely to death.<sup>(7)</sup>

Antigen detection tests and culture are used in laboratories for diagnosing Influenza and RSV infection, RT-PCR assays have been shown to be a more accurate and reliable tool for the detection of Influenza A, Influenza B and RSV viruses.

## **Principles of the Procedure**

The Savvygen™ Flu A, Flu B & RSV assay is designed for detection of Influenza A, Influenza B and/or Respiratory Syncytial Viruses in respiratory specimens to aid in the assessment of infections caused by these pathogens.

The Savvygen™ Flu A, Flu B & RSV test is based on amplification of highly conserved fragments in the *M1* gene (Flu A and Flu B) and in the *N* gene (RSV). Following extraction of viral RNA, the conserved fragments are reversely transcribed into cDNA in a primer-specific manner (Figure 1a). Reverse transcription is followed in a “one-pot reaction” by Taq Polymerase Chain Reaction (PCR). The assay is based on the 5'→3' exonuclease activity of Taq DNA Polymerase (Figure 1b). A fluorophore/quencher dual-labeled probe is annealing to an internal specific sequence. Upon primer elongation, Taq DNA Polymerase displaces and hydrolyzes the probe, thus releasing and activating the fluorophore. The presence of Flu A, Flu B and RSV is detected by an increase in observed fluorescence during the reaction. The resulting increase in fluorescence signal is proportional to the amount of amplified product in the sample and detected by the real-time PCR thermocycler.



**Figure 1.** Principle of the Savvygen assay

The Savvygen™ Flu A, Flu B & RSV test is a ready-to-use assay containing in each well all the necessary reagents for the reaction in a stabilized format. An internal control allows the identification of a possible inhibition of the reaction. The optical channels used for multiplexed detection of the amplified fragments are outlined in table 1 below:

**Table 1.** Savvygen™ Flu A, Flu B & RSV viruses target and optical channel detection

Target	Optical channel
Influenza A	FAM
Influenza B	ROX
RSV	Cy5
Internal Control	*HEX, VIC or JOE

(\*) Depending on the equipment used, the proper detection channel should be selected (see table 4)

## Materials/ Reagents Provided

Product Description	Contents
<b>Savvygen™ Flu A, Flu B &amp; RSV</b> 48 reactions. Cat.# 612-01H / 612-01L	6 x Savvygen™ Flu A, Flu B & RSV strips
	1x Savvygen™ Flu A, Flu B & RSV Positive Control
	1x Water RNase/DNase free 1mL
	1x Rehydration Buffer 1.8 mL
	1x Negative Control 1 mL
	Optical caps

## Additional Equipment and Material Required

- RNA extraction kit.
- Centrifuge for 1.5 mL tube.
- Vortex.
- Micropipettes (0.5-20 µL, 20-200 µL).
- Powder-free disposal gloves
- Real Time PCR instrument (see table 2A+2B for compatible RT-PCRs).

**Table 2A.** Compatible Real Time PCR instruments (Low-Profile)

<b>Bio-Rad</b>	<b>Applied Biosystems</b>
CFX96 Touch™ Real-Time PCR Detection System	7500 Fast Real-Time PCR System
<b>Roche</b>	7500 Fast Dx Real-Time PCR System
LightCycler®480 Real-Time PCR System	QuantStudio™ 12K Flex 96-well Fast
LightCycler®96 Real-Time PCR System	QuantStudio™ 6 Flex 96-well Fast
<b>Agilent Technologies</b>	QuantStudio™ 7 Flex 96-well Fast
AriaMx Real-Time PCR System	QuantStudio™ 5 Real-Time PCR System
<b>DNA-Technology</b>	ViiA™ 7 Fast Real-Time PCR System
DTlite Real-Time PCR System	<b>Cepheid</b>
DT prime Real-Time Detection Thermal Cycler	SmartCycler®*
	<b>Qiagen</b>
	Rotor-Gen® Q*

**Table 2B.** Compatible Real Time PCR instrument (High-Profile)

<b>Bio-Rad</b>	<b>Applied Biosystems</b>
<i>CFX96 Touch Deep Well Real-Time PCR Detection System</i>	<i>7500 Real-Time PCR System</i>
<i>iCycler iQ Real-Time PCR Detection System</i>	<i>QuantStudio™ 12K Flex 96-well</i>
<i>iCycler iQ 5 Real-Time PCR Detection System</i>	<i>QuantStudio™ 6 Flex 96-well</i>
<b>DNA-Technology</b>	<i>QuantStudio™ 7 Flex 96-well</i>
<i>DTlite Real-Time PCR System</i>	<i>QuantStudio™ 5 Real-Time PCR System</i>
<i>DT prime Real-Time Detection Thermal Cycler</i>	<i>ViiA™ 7 Real-Time PCR System</i>
<b>Stratagene /Agilent Technologies</b>	<b>Qiagen</b>
<i>Mx3000P™ Real Time PCR System</i>	<i>Rotor-Gen® Q*</i>
<i>Mx3005P™ Real Time PCR System</i>	<b>Cepheid</b>
<b>Analytik Jena Biometra</b>	<i>SmartCycler®*</i>
<b>TOptical</b>	<b>Abbot</b>
<i>qTOWER 2.0</i>	<i>Abbot m2000 RealTime System</i>

\* Once the product has been reconstituted following the appropriate procedure, transfer it in the specific Rotor-Gene® Q or SmartCycler® tubes.

## Precautions

Amplification technologies can amplify target nucleic acid sequences over a billion-fold and provide a means of detecting very low concentrations of target. Care must be taken to avoid contamination of samples with target molecules from other samples, or amplicons from previous amplifications. Follow these recommendations to help control contamination.

1. Separate pre-amplification steps from post-amplification steps. Use separate locations for pre- and post-amplification. Use dedicated lab equipment for each stage. Prepare samples in a laminar flow hood using dedicated equipment to minimize contamination. Set up the post-amplification area in a low-traffic area with dedicated equipment.
2. The laboratory process must be one-directional, it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.
3. Use disposable containers, disposable barrier pipette tips, disposable bench pads, and disposable gloves. Avoid washable lab wear.
4. Use a diluted bleach solution (0.2% sodium hypochlorite) to treat waste from the post-amplification and detection areas, as the waste contains amplicon. Use the bleach solution to wipe down equipment and bench areas, and to treat drains used to dispose of liquid waste.
5. Use negative controls to monitor for possible contamination during reaction setup. If reagent contamination is detected, dispose of the suspect reagents.
6. Do not use after expiration date.
7. Specimens must be treated as potentially infectious as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take necessary precautions during the collection, storage, treatment and disposal of samples.



## ***Transport and Kit Storage***

The Savvygen kits can be shipped and stored at 2-37°C until expiration date stated in the label.

After resuspension of the Positive Control, store at -20°C. Avoid repeated freeze/thaw cycles.

It is recommended to make aliquots of the positive control and stored at -20°C once resuspended in order to avoid freeze & thaw cycles.

## ***Test Procedure***

### **Specimen Collection, Processing and RNA Extraction**

In order to obtain an adequate sample, the procedure for sample collection must be followed closely and according to the manufacturer's instructions. The specimens should be transported as fast as possible and to be stored at the indicated temperatures conditions.

**Nucleic Acid (NA) Extraction:** for pretreatment and NA isolation, it is recommended to use an appropriate RNA extraction kit according to manufacturer's protocol. NA Extraction may be carried out manually or automatically using commercially available extraction kits. Several extraction systems were validated for this kit including:

- Savvygen Extractor (Savyon Diagnostics)
- Maxwell®16 Viral Total Nucleic Acid Purification Kit, using the Maxwell® 16 instrument (Promega).
- Total Nucleic Acid Isolation (TNAI) Kit, using COBAS® AmpliPrep (Roche).
- RIDA® Xtract (r-Biopharm).
- Qiagen® Viral RNA kit

### **Positive Control Preparation**

**Note:** The Positive Control vial contains high copies number template of the assay targets with a contamination risk. Therefore, it is recommend resuspend the vial in a separate laboratory area or a special cabinet.

Open the Positive control pouch to resuspend the lyophilized *Flu A*, *Flu B* & *RSV* Positive Control (tube of red cap) with 100 µL of Water RNase/DNase free (transparent cap vial) supplied. To ensure a complete resuspension, vortex the tube thoroughly. After first use, dispense into aliquots in order to avoid multiple freeze-thaw cycles, and store them at -20°C.

### **PCR Protocol Program**

Set your thermocycler following the conditions below:

**Table 3.** Real time RT-PCR profile

Step	Temperature	Time	Cycles
Reverse transcription	45°C	15 min	1
Initial denaturation	95°C	2 min	1
Denaturation	95°C	10 sec.	45
Annealing/Extension*	60°C	50 sec.	

**Note:** Set the fluorescence data collection during the extension step (\*) through the FAM (Flu A), ROX (Flu B), Cy5 (RSV) and HEX, JOE or VIC channels (Internal Control (IC)). Depending on the equipment used select the proper detection channel (table 4). For the Applied Biosystems 7500 Fast Real-Time PCR system check that passive reference option ROX is not marked.

### Preparing Reaction Wells

#### **A. Reconstitute the required reaction wells.**

Calculate the number of required reactions including samples and controls. It is highly recommended to run at least one positive and one negative control per run.

1. Peel off protective aluminum seal from the strips/plate and
2. Pipette 15 µL of Rehydration Buffer (Blue cap vial) into each well.

#### **B. Add samples and controls according to real-time PCR experimental plate set up.**

1. Pipette 5 µL of RNA sample into each sample well.
2. Pipette 5 µL of resuspended *Flu A*, *Flu B* & RSV Positive Control (tube of red cap) into each positive control well.
3. Pipette 5 µL of Negative Control (tube of orange cap) into each negative control well.
4. Cover the wells with the caps provided. Spin down briefly if needed.

#### **C. Performing PCR.**

1. Place the strips in the Real Time PCR instrument.
2. Start the run.

The fluorescence detection channels of common Real Time PCR Thermocyclers are specified in Table 4.

**Table 4:** Detection fluorescence channels of different Real Time PCR systems

<b>RT- PCR THERMOCYCLER</b>	<b>System Detection channels</b>	<b>Savvygen probes channels</b>	<b>Remarks</b>
<b>Roche LightCycler® 96 or LightCycler®480II</b>	465/510	FAM	Color Compensation is required only for LC480 system
	533/580	HEX	
	533/610	ROX	
	618/660	Cy5	
<b>Applied Biosystems ABI 7500 fast</b>	FAM	FAM	Passive reference option ROX is not mark
	VIC	HEX	
	ROX	ROX	
	Cy5	Cy5	
<b>Bio-Rad CFX96™</b>	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
<b>Agilent AriaMx</b>	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	
<b>DNA-Technology DTlite / DTprime</b>	FAM	FAM	
	HEX	HEX	
	ROX	ROX	
	Cy5	Cy5	

## Interpretation of results

Interpretation of results can be automatically performed if programmed by the user using the RT-qPCR instrument software following manufacturer's instructions. It is required to run assay controls (positive and negative controls) in each run to validate the reaction.

**Note:** The positive controls used in each run, must show an amplification curve of the tested targets (Flu A, Flu B & RSV) which validates the reaction, while the negative control well should demonstrate an absence of signal (except internal control target).

The result interpretation is done according to Table 5.

**Positive sample-** A sample is considered a positive, if the obtained amplification curve signal is below threshold cycle 40.

**Negative sample-** A sample is considered negative for the target if there is no evidence of amplification curve signal in the detection system and the internal control has a positive signal amplification.

**Internal control-** The Internal Controls must show an amplification curve, which verify the correct functioning of the amplification mix. It should be noted that the detection of internal control may not be necessary in case of positive result of the target sequence.

**Positive control-** The positive controls used in each run, must show amplification curves for Flu A, Flu B and RSV which validates the reaction.

**Negative control-** The negative controls included in each run, must show an absence of signal for Flu A, Flu B or RSV which validates the reaction.

**Invalid run-** The assay should be considered as invalid and a new run should be performed if there is signal of amplification for one of the pathogens in the negative control well or absence of signal in the positive control well.

**Note:** If an amplification curve for the internal control is not shown, it may be due to PCR inhibitors. In such case the sample should be retested by dilution of the original sample 1:10. Alternatively it is recommended to repeat the nucleic acid extraction.

**Table 5.** Results interpretation

Flu A (FAM)	Flu B (ROX)	RSV (Cy5)	Internal control (HEX, VIC or JOE)	Negative Control	Positive Control	Interpretation
POS	POS	POS	POS / NEG	NEG	POS	Flu A, Flu B and RSV Positive
NEG	NEG	NEG	POS	NEG	POS	Flu A, Flu B and RSV Negative
POS	NEG	NEG	POS / NEG	NEG	POS	Flu A Positive, Flu B and RSV Negative
NEG	POS	NEG	POS / NEG	NEG	POS	Flu B Positive, Flu A and RSV Negative
NEG	NEG	POS	POS / NEG	NEG	POS	RSV Positive, Flu A and Flu B Negative
POS	POS	NEG	POS / NEG	NEG	POS	Flu A and Flu B Positive, RSV Negative
NEG	POS	POS	POS / NEG	NEG	POS	Flu B and RSV Positive, Flu A Negative
POS	NEG	POS	POS / NEG	NEG	POS	Flu A and RSV Positive, Flu B Negative
NEG	NEG	NEG	NEG	NEG	POS	Invalid run
POS	POS	POS	POS	POS	POS	Invalid run

**POS:** presents of amplification signal

**NEG:** no amplification signal



## Limitations of the test

- All results should be used and interpreted in the context of a full clinical evaluation as an aid in the diagnosis of respiratory tract infection.
- This assay was only validated for nasal, nasopharyngeal and throat swabs samples. The use of other samples has not been established.
- Error results may occur from improper sample collection, handling, storage, technical error, sample mix-up, or because the number of organisms in the sample is below the analytical sensitivity of the test.
- The presence of PCR inhibitors may cause invalid results.
- A false positive result with other targets is possible due to contamination with PCR products from previous testing.
- As with all PCR-based diagnostic tests, extremely low levels of target below the analytical sensitivity of the assay may be detected, but results may not be reproducible.
- If a certain sample result is Invalid then the sample should be repeated from RNA extraction.

## Quality Control

In order to confirm the appropriate performance of the molecular diagnostic technique, an Internal Control (IC) is included in each reaction. Besides, a positive and a negative control must be included in each assay to interpret the results correctly.

## Performance Characteristics

### Clinical Sensitivity and Specificity

Clinical performance characteristics of the Savvygen™ Flu A, Flu B & RSV test were assessed in clinical study performed in Israel (Meir hospital, Kfar-Saba) by evaluation of clinically-obtained retrospective (frozen) specimens. Study specimens consisted of 191 nasal pharyngeal swabs (UTM™, Universal Transport Medium; Copan Diagnostics) from patients with respiratory symptoms.

Specimens were characterized by the source site's routine laboratory methodologies and included molecular testing (Simplexa™ Flu A/B & RSV; Focus Diagnostics) and in-house assay which comprised the reference method at winter and summer time. Discrepant results analysis was performed by further investigation of the sample in qRT-PCR (Flu/HRSV kit; Fast-Track Diagnostics) assay. Viral RNA was extracted using Savvygen Extractor (Distributed by Savyon Diagnostics) or the NucliSENS easyMag (bioMerieux, as well as with manual Viral RNA extraction kit (Qiagen). All extracts were stored at -20°C before and after use. Results appear in table 6.

**Table 6.** Clinical study results

Pathogen	Positive Agreement		Specificity	
	TP/ (TP+FN)	Percent	TN/ (TN+FP)	Percent
<b>Influenza A</b>	54/56	96.40%	98/99	100%
<b>Influenza B</b>	52/52	100%	98/98	100%
<b>RSV</b>	40/41	97.6%	109/110	100%

In a second clinical study performed in Spain, a total of 187 respiratory frozen samples from symptomatic patients, were tested by the Savvygen™ Flu A, Flu B & RSV test versus a reference assay (CLART® PneumoVir; Genomica). Results are shown in table 7.

**Table 7.** Clinical study results

Pathogen	Positive Agreement		Specificity	
	TP/ (TP+FN)	Percent	TN/ (TN+FP)	Percent
<b>Influenza A</b>	72/74	96.2%	113/113	100.0%
<b>Influenza B</b>	43/44	97.7%	143/143	100.0%
<b>RSV</b>	36/36	100.0%	151/151	100.0%

### Analytical sensitivity

A serial dilution test was conducted to evaluate the analytical sensitivity of the Savvygen™ Flu A, Flu B & RSV for each pathogen- *Influenza A*, *Influenza B* and Respiratory syncytial virus. The Savvygen™ Flu A, Flu B & RSV has presented a detection limit of  $\geq 10$  viral RNA copies per reaction (Figure 2, 3 and 4).

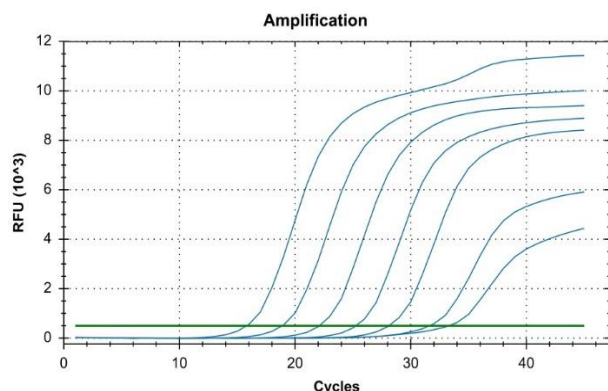


Figure 2. Amplification plot for 10-fold dilution series of Flu A template ranging from 10<sup>7</sup> to 10<sup>1</sup> copies/reaction (FAM channel).

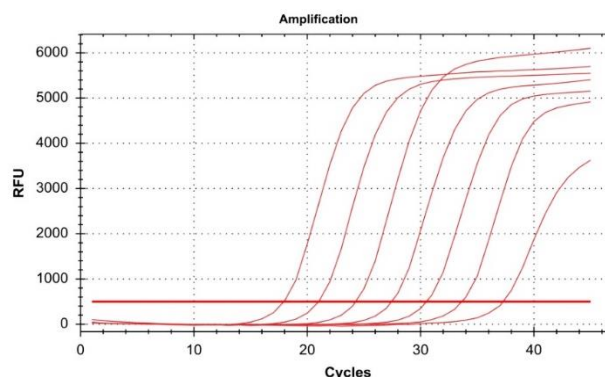


Figure 3. Amplification plot for 10-fold dilution series of Flu B template ranging from 10<sup>7</sup> to 10<sup>1</sup> copies/reaction (ROX channel).

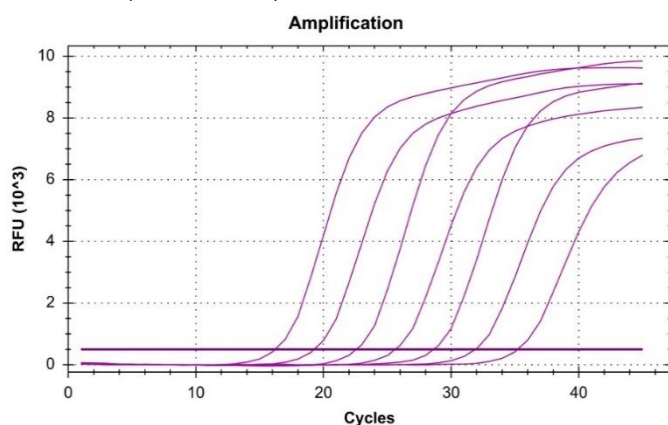


Figure 4. Amplification plot for 10-fold dilution series of RSV template ranging from 10<sup>7</sup> to 10<sup>1</sup> copies/reaction (Cy5 channel).

### **Analytical specificity**

Cross-reactivity testing was conducted with the Savvygen™ Flu A, Flu B & RSV to assess the analytical specificity of the test with most common respiratory pathogens. As shown in table 8, *no* cross-reactivity was observed for Flu A, Flu B or RSV with other respiratory microorganisms.

**Table 8. Cross-reactivity testing.**

Pathogen	Cross-Reactivity Test		
	Savvygen™ Flu A, Flu B & RSV		
	Influenza A	Influenza B	Respiratory syncytial virus
<i>Bordetella pertussis</i>	-	-	-
<i>Haemophilus influenza</i>	-	-	-
Human Adenovirus	-	-	-
Human coronavirus 229E	-	-	-
Human metapneumovirus A and B	-	-	-
Human parainfluenza 1, 2, 3 and 4 viruses	-	-	-
Human rhinovirus	-	-	-
Influenza A/California/7/2009(H1N1) virus	+	-	-
Influenza A/Perth/16/2009(H3N2) virus	+	-	-
Influenza A/New Caledonia/20/99(H1N1) virus	+	-	-
Influenza A/Switzerland/9715293/2013	+	-	-
Influenza A/Turkey/Germany R2485+86/2014	+	-	-
Influenza B/Brisbane/60/2008 virus	-	+	-
Influenza B/Florida/04/06 virus	-	+	-
Influenza B/Phuket/3073/2013	-	+	-
<i>Legionella bozemanii</i>	-	-	-
<i>Legionella micdadei</i>	-	-	-
<i>Legionella dumoffii</i>	-	-	-
<i>Legionella longbeachae</i>	-	-	-
<i>Legionella pneumophila</i>	-	-	-
MERS-Coronavirus	-	-	-
Methicillin-resistant <i>Staphylococcus aureus</i>	-	-	-
<i>Mycoplasma pneumoniae</i>	-	-	-
<i>Moraxella catarrhalis</i>	-	-	-
Respiratory syncytial virus (RSV)	-	-	+
<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	-	-	-
<i>Streptococcus pneumoniae</i>	-	-	-

### **Analytical reactivity**

The reactivity of Savvygen™ Flu A, Flu B & RSV test was evaluated versus different strains of Influenza A, Influenza B and Respiratory syncytial virus respectively (table 9). All strains of Influenza A, Influenza B and Respiratory syncytial virus were detected by the assay accordingly.

**Table 9.** Analytical reactivity test of the Savvygen™ Flu A, Flu B & RSV with Influenza A, B and RSV strains

Pathogen	Analytical-Reactivity Test		
	Savvygen™ Flu A, Flu B & RSV		
	Influenza A	Influenza B	Respiratory syncytial virus
Influenza A/California/7/2009(H1N1)	POS	NEG	NEG
Influenza A/Perth/16/2009(H3N2)- like	POS	NEG	NEG
Influenza A/New Caledonia/20/99(H1N1)-like	POS	NEG	NEG
Influenza A/Switzerland/9715293/2013	POS	NEG	NEG
Influenza A/Turkey/Germany R2485+86/2014	POS	NEG	NEG
Influenza A/Michigan/45/2015 (H1N1)pdm09	POS	NEG	NEG
Influenza A/Thüringen/5/17 (H3N2)	POS	NEG	NEG
Influenza A/Anhui/1/2013 (H7N9)	POS	NEG	NEG
Influenza A/DE-SH/Reiherente/AR8444/ 2013 (H5N8)	POS	NEG	NEG
Influenza B/Brisbane/60/2008- like	NEG	POS	NEG
Influenza B/Florida/04/06 virus	NEG	POS	NEG
Influenza B/Phuket/3073/2013	NEG	POS	NEG
Respiratory syncytial virus (RSV)	NEG	NEG	POS



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