Instructions for Use

LyteStar™
Influenza Typing RT-PCR Kit
1.0

LyteStar™
Influenza Typing RT-PCR Kit 1.0

For detection of Influenza A and Influenza B virus and typing of Influenza A(H1N1)pdm09 and H3N2 from human specimens

For use with

CFX96™ (BioRad)
CFX Opus 96 (BioRad)

IVD For *in vitro* diagnostic use

REF Product No.: 890003

96 reactions

Please refer to Storage and Shelf Life in this IFU

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Content

1. Intended Use

The LyteStar™ Influenza Typing RT-PCR Kit 1.0 is intended for the specific detection of Influenza A/B RNA in human respiratory specimens including deep nostrils (nasal swab), throat (oropharyngeal swab) and nasopharyngeal swabs placed in VTM, nasopharyngeal wash/aspirate, nasal wash/aspirate and bronchial aspirate. The LyteStar™ Influenza Typing RT-PCR Kit 1.0 is a four-target assay comprising a screening assay targeting the Influenza A matrix *(M)* and Influenza B hemagglutinin *(HA)* genes. The assay further differentiates the Influenza A into A(H1N1)pdm09 and H3N2, via the *HA* gene.

The LyteStar™ Influenza Typing RT-PCR Kit 1.0 is for professional use only.

2. Kit Components

Catalog no.	890003
Master A	2 x 300 µl
Master B	4 x 330 µl
Internal Control (IC)	800 µl
Positive Control (PC)	200 µl
PCR grade water	500 µl

3. Storage and Shelf Life

- The LyteStar™ Influenza Typing RT-PCR Kit 1.0 has a shelf life of 12 months from the manufacturing date.
- Store all reagents at -15°C to -25°C upon arrival.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay. Master B should be frozen in aliquots, if they are to be used intermittently.
- Mix Master A thoroughly by vortex mixing, and centrifuge briefly before use.
- Protect Master B from light.
- All frozen reagents should be completely thawed to room temperature before use. Immediately return unused portions to the freezer for storage.

4. Quality Control

In compliance with AstronDX Technologies' EN ISO 13485 certified Quality Management System, each lot of the LyteStar™ Influenza Typing RT-PCR Kit 1.0 is tested against pre-determined specifications to ensure consistent product quality.

5. Product Use Limitations and Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR procedures.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNAse/RNAse) contamination of the specimen and the components of the kit.
- Always use DNAse/RNAse-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) specimen preparation, (ii) reaction set-up and (iii) amplification/detection activities.
- Workflow in the laboratory should proceed in unidirectional manner.
- Always wear disposable gloves in each area and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

- Wash hands thoroughly after handling specimens and test reagents.
- Do not use kits from different lots together.
- Do not use an expired kit.
- In case of damage to the packaging and leaking vials, do not use the kit (possible contamination or deterioration that can cause false interpretation).

6. Product Warranty

AstronDX Technologies guarantees the performance of the LyteStar™ Influenza Typing RT-PCR Kit 1.0 for applications as described in the manual. The user must determine the suitability of the product for the particular intended use. Should the product fail to perform satisfactorily in the described applications, please contact AstronDX Technologies Technical Support (**16. Technical Support**) for trouble-shooting.

AstronDX Technologies reserves the right to change, alter, or modify any product to enhance its performance and design.

7. Product Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles/face masks. For more information, please consult the appropriate material safety data sheets (MSDSs).

8. Introduction

Influenza A and B viruses are responsible for seasonal flu epidemics, more commonly known as the flu season. Influenza viruses cause a contagious respiratory illness that infects the nose, throat, and lungs. Infected individuals often experience some or all of these symptoms; fever, cough, sore throat, runny nose, body aches, headaches, fatigue, vomiting, and in some cases, diarrhoea. Although most people could recover from symptoms within a week without treatment, for some, Influenza may cause severe illness, which may lead to death in people with higher risk such as pregnant women, children below 5 years old, elderly above 65 years old, and people with chronic disease and complications. Globally, the annual epidemics of Influenza are estimated to result in about 3-5 million cases of severe illness, and about 290,000-650,000 respiratory deaths [1,2].

Influenza A viruses can be further classified into subtypes due to the shuffling between the hemagglutinin (HA) and the neuraminidase (NA), the major surface antigens of the virus. Currently, the most predominant circulating subtypes in

humans are the A(H1N1)pdm09 and A/H3N2 Influenza viruses. Influenza B viruses can be divided into lineages B/Yamagata and B/Victoria. Influenza C is detected less frequently with mild infections while Influenza D viruses are known to primarily infect cattle. Thus, both Influenza C and D do not present public health importance [2,3].

The respiratory illness caused by Influenza viruses is difficult to distinguish from other circulating respiratory viruses that present Influenza-like illnesses such as SARS-CoV-2, rhinovirus, adenovirus, respiratory syncytial virus, and parainfluenza (and vice-versa), hence making a diagnosis of Influenza, or differentiating other respiratory infection from Influenza, based on clinical presentation alone difficult. Patients with severe or progressive symptoms associated with confirmed Influenza infection such as pneumonia, sepsis, or worsening of chronic underlying diseases should be treated with an antiviral drug (Oseltamivir: neuraminidase inhibitor) ideally within 48 hours following symptom onset to maximize therapeutic benefits [3].

Although safe and effective inactivated Influenza vaccines are available, the immunity or protection from vaccination subsides over time. Therefore, annual vaccination is recommended to protect against Influenza infection, especially for those at high risk of Influenza complications. Due to the constantly evolving nature of Influenza viruses, there is a need for the WHO Global Influenza Surveillance and Response System (GISRS) to continuously monitor the circulating Influenza viruses in humans and revise the composition of vaccines twice a year to improve the effectiveness of vaccines. This highlights the importance of subtyping the Influenza viruses since accurate epidemiological data is crucial to facilitate timely guidance for appropriate antiviral, chemoprophylaxis use, and annual vaccine production [3].

In response to this changing paradigm in Influenza diagnosis and treatment, nucleic acid amplification tests are preferred because of the speed, sensitivity, and ability to subtype Influenza viruses directly on clinical specimens. Various real-time RT-PCR assays have been published to detect seasonal Influenza. The LyteStar™Influenza Typing RT-PCR Kit 1.0 was developed based on four assays previously described [4]. The assays target the *M* gene (Screening for Influenza A), *HA* gene (Screening for Influenza B), *HA* gene (Subtyping for Influenza A(H1N1)pdm09), and *HA* gene (Subtyping for Influenza A/H3N2).

[1] Wang X, Li Y, O'Brien KL, Madhi SA, Nair H; Respiratory Virus Global Epidemiology Network. Global burden of respiratory infections associated with seasonal influenza in children under 5 years in 2018: a systematic review and modeling study. Lancet Glob Health. 2020 Apr;8(4): e497-e510.

[2] Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases (NCIRD) 2022. Available online https://www.cdc.gov/flu/about/viruses/types.html (last cited: 13 January 2023).

[3] WHO Influenza (seasonal) Geneva: World Health Organization 2023. Available online https://www.who.int/news-room/fact-sheets/detail/influenza (seasonal)# (last cited: 13 January 2023).

[4] WHO information for the molecular detection of influenza viruses: World Health Organization 2021. Available online https://cdn.who.int/media/docs/default-source/influenza/molecular-detention-of-influenzaviruses/protocols_influenza_virus_detection_feb_2021.pdr?sfvrsn=df7d268_5# (Protocol 3, Page:66)(last cited: 25 January 2023).

9. Product Description

The LyteStar™ Influenza Typing RT-PCR Kit 1.0 is an *in vitro* diagnostic test system, based on real-time PCR technology, for the qualitative detection and differentiation of Influenza A, Influenza B, Influenza A(H1N1), and Influenza A H3N2 specific RNA. The LyteStar™ Influenza Typing RT-PCR Kit 1.0 consists of four assays, one targeting the *M* gene for identification of Influenza A, the second targeting the *HA* gene that specifically detects Influenza B and two more assays targeting the Influenza A *HA* gene to differentiate between the Influenza A(H1N1)pdm09 and Influenza A/H3N2. The LyteStar™ Influenza Typing RT-PCR Kit 1.0 includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences, and target-specific probes for the detection of the amplified DNA. The probes are labeled with a fluorescent reporter and quencher dyes. The probes used for specific amplification of Influenza A, B, A(H1N1)pdm09, and A/H3N2 RNA are labeled with the fluorophore Tye705™, TEX615™, Cy5™, and FAM™, respectively. The probe specific to the target of the Internal Control (IC) is labelled with the fluorophore HEX™. Using probes linked to distinguishable dyes enables the parallel detection and differentiation of Influenza specific RNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The oligonucleotides in the four assays were designed based on sequences/target regions published in the articles listed below:

Target	Publication
Influenza A	Van *et al*., (2005) and Duchamp *et al*., (2009)
Influenza B	Van *et al*., (2001)
Influenza A(H1N1)pdm09	Mina *et al*., (2011)
Influenza A/H3N2	
Internal Control	Deer *et al*., (2010)

The test consists of three processes in a single tube assay:

- Reverse transcription of target and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The LyteStar™ Influenza Typing RT-PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- The template of the Internal Control (IC)
- The template of the Positive Control (PC)
- PCR grade water (for setting up of “No Template Control”, NTC)

Master A and Master B reagents contain all components (buffer, enzymes, primers and probes) to allow PCR mediated reverse transcription, amplification and target detection of *M* gene of Influenza A specific RNA, *HA* gene for specific detection and differentiation of Influenza B, A(H1N1)pdm09 and A/H3N2, and Internal Control in one reaction setup.

The Positive Control (PC) contains *in vitro* transcripts of synthesized target genes of Influenza A, Influenza B, A(H1N1)pdm09, and A/H3N2.

The Internal Control used in the LyteStar™ Influenza Typing RT-PCR Kit 1.0 is an *in vitro* transcribed RNA of an artificial sequence with no homology to any known genomes.

The LyteStar™ Influenza Typing RT-PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- CFX96™ (BioRad)
- CFX Opus 96 (BioRad)

10. Material and Devices required but Not Provided

- Appropriate real-time PCR instrument
- Appropriate nucleic acid extraction system or kit
- 1.5 ml microcentrifuge tubes (with safe-lock or screw cap)
- Microcentrifuge (with speed ≥ 13,000 rpm)
- Pipettes, adjustable (range: 10 µl, 100 µl, 200 µl, 1000 µl)
- Pipette tips (with aerosol barriers)
- Disposable gloves (powder-free)
- Heating block for lysis of specimens during extraction
- Vortex mixer
- Appropriate 96-well reaction plates or reaction tubes with corresponding (optical) closing material **(Clear tubes are recommended. Do not use white tubes).**

11. Specimen Storage

- Suitable specimens include deep nostrils (nasal swab), throat (oropharyngeal swab) and nasopharyngeal swabs placed in VTM, nasopharyngeal wash/aspirate, nasal wash/aspirate and bronchial aspirate.

- Follow specimen transport and storage conditions outlined in the following guidelines:

  - WHO Global Influenza Surveillance Network. Manual for the laboratory diagnosis and virological surveillance of influenza (https://apps.who.int/iris/handle/10665/44518), version 2011
  - CDC Centre for Disease Control and Prevention. Influenza Specimen Collection. https://www.cdc.gov/flu/pdf/freeresources/healthcare/flu-specimen-collection-guide.pdf

12. Instructions for Use

12.1. Sample Preparation

Extracted RNA is the starting material for the LyteStar™ Influenza Typing RT-PCR Kit 1.0. The quality of the extracted RNA has a profound impact on the performance of the whole test system. It has to be ensured that the nucleic acid extraction system used is compatible with real-time PCR technology.

The following nucleic acid extraction kits / systems are suitable for use with the LyteStar™ Influenza Typing RT-PCR Kit 1.0:

- SpinStar™ Viral Nucleic Acid Kit (AstronDX Technologies)
- MagCore® Plus II Automated Nucleic Acid Extractor (RBC Bioscience)
- QIAamp® MinElute Virus Spin Kit (Qiagen)
- QIAamp® Viral RNA Mini Kit (Qiagen)
- QIAsymphony® (Qiagen)
- HighPure® Viral Nucleic Acid Kit (Roche)
- NucliSENS® easyMag (bioMérieux)
- MagNA Pure 96 System (Roche)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with LyteStar™ Influenza Typing RT-PCR Kit 1.0 has to be validated by the user.

If using a spin column-based sample preparation procedure including washing buffers containing ethanol, an additional centrifugation step for 10 min at approxiately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid is highly recommended.

NOTE

Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.

12.2. Master Mix Setup

1. All reagents and samples should be thawed completely, mixed (by gentle vortex mixing) and centrifuged briefly before use. Prepare a marginal excess (additional 0.5 reaction) of the required Master Mix volume.

2. The LyteStar™ Influenza Typing RT-PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as (i) a PCR inhibition control or

as (ii) a control of the sample preparation procedure (nucleic acid extraction) and PCR inhibition control.

(i) If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions	1	12
Master A	6.25 µl	75 µl
Master B	13.75 µl	165 µl
IC	0.5 µl	6 µl
Volume Master Mix	**20.5 µl**	**246 µl**

(ii) If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, the IC has to be added during the nucleic acid extraction procedure.

No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture.

The volume of the IC which has to be added depends always and only on the elution volume. It represents **10% of the elution volume**. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into the specimen/lysis buffer mixture.

If the IC was added during the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions	1	12
Master A	6.25 µl	75 µl
Master B	13.75 µl	165 µl
Volume Master Mix	**20 µl**	**240 µl**

NOTE

⚠ ***Never add the Internal Control directly to the specimen.***

12.3. Reaction Setup

1. Pipette 20 µl Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.

2. Add 5 µl of the sample (eluate from the nucleic acid extraction) or 5 µl of the controls (Positive Control; or water as No Template Control, NTC).

3. Make sure at least one Positive Control and one NTC are used per run.

4. Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.

5. Close the 96-well reaction plate with an appropriate optical adhesive film and the reaction tubes with appropriate caps.

6. Centrifuge the 96-well reaction plate at 1,000 x g (~3,000 rpm) for 30s.

Reaction Setup	
Master Mix	20 µl
Sample or Control	5 µl
Total Volume	**25 µl**

13. Programming the Real-Time PCR Instrument

13.1 Settings

• Define the following settings:

Settings	
Reaction Volume	25 µl
Ramp Rate	Default

13.2 Fluorescent Detectors (Dyes)

• Define the following fluorescent detectors:

Detection	Detector Name	Reporter	Quencher
Influenza A (*M* gene) specific RNA	Flu A	Quasar705	BHQ1
Influenza B (*HA* gene) specific RNA	Flu B	Tex615	IBRQ
Influenza A (H1N1)pdm09 (*HA* gene) specific RNA	Flu A(H1N1) pdm09	Cy5	IBRQ
Influenza A/H3N2 (*HA* gene) specific RNA	Flu A/H3N2	FAM	IBRQ
Internal Control	IC	HEX	IBFQ

13.3 Temperature Profile and Dye Acquisition

• Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Reverse-transcription	Hold	1	-	50 °C	10:00 min
Denaturation	Hold	1	-	95 °C	2:00 min
Amplification	Cycling	45	-	95 °C	5 sec
			√	55 °C	30 sec

√ Signal acquisition: activate FAM, Cy5, Tex615, Quasar705 and HEX channels in all runs.

14. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument. For detailed instructions regarding data analysis of the LyteStar™ Influenza Typing RT-PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (**16.Technical Support**).

14.1. Validity of Diagnostic Test Runs

14.1.1 Valid Diagnostic Test Runs (Qualitative)

For a **valid** diagnostic test run (qualitative), the following control conditions must be met:

Control ID	**FAM/Cy5/Tex615 Quasar705** Detection Channel	**HEX** Detection Channel
Positive Control	POSITIVE	POSITIVE
Negative Control	NEGATIVE	POSITIVE

14.1.2 Target CT values of PC and IC

	Positive Control *(Flu A)*	**Positive Control** *(Flu B)*	**Positive Control** *(Flu A (H1N1) pdm09)*	**Positive Control** *(Flu A/H3N2)*	**Internal Control**
Target CT value	< 35 cycles	< 35 cycles	< 35 cycles	< 35 cycles	≤ 40 cycles*

*Required for unknown samples that do not amplify in FAM, Cy5, Tex615, and Quasar705 channels

Note: The above CT target values are exclusively given for **monitoring the integrity of the product and validated assay conditions** and should be achieved **ONLY for the provided Positive Control (PC) and Internal Control (IC)** when used as per the instructions given under section 12.3. Reaction set up. **The target CT values for PC MUST NOT be misinterpreted as the diagnostic cut-off values for clinical samples.**

14.1.3 Invalid Diagnostic Test Runs (Qualitative)

A **qualitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, **repeat testing by using the remaining purified nucleic acids** or start from the original samples again.

14.2 Interpretation of Results

Quasar 705 *Flu A*	**Tex615** *Flu B*	**Cy5** *Flu A (H1N1) pdm09*	**FAM** *Flu A/H3N2*	**HEX** *Internal Control*	**Result Interpretation**
+	+	+	+	+*	Influenza A, Influenza B, Influenza A(H1N1)pdm09 and Influenza A/H3N2 specific RNA detected. *Positive for Influenza A, Influenza B, Influenza A(H1N1)pdm09 and Influenza A/H3N2*
+	+	+	-	+*	Influenza A, Influenza B and Influenza A(H1N1)pdm09 specific RNA detected. *Positive for Influenza A, Influenza B and Influenza A(H1N1)pdm09*
+	+	-	+	+*	Influenza A, Influenza B and Influenza A/H3N2 specific RNA detected. *Positive for Influenza A, Influenza B, and Influenza A/H3N2*
+	-	+	+	+*	Influenza A, Influenza A(H1N1) pdm09 and Influenza A/H3N2 specific RNA detected. *Positive for Influenza A, Influenza A(H1N1)pdm09 and Influenza A/H3N2*
-	+	+	+	+*	Influenza B, Influenza A (H1N1) pdm09 and Influenza A/H3N2 specific RNA detected. *Positive for Influenza B, Influenza A(H1N1)pdm09 and Influenza A/H3N2* [B,C]

+	+	-	-	+*	Influenza A and Influenza B specific RNA detected. *Positive for Influenza A and Influenza B*
+	-	+	-	+*	Influenza A and Influenza A (H1N1)pdm09 specific RNA detected. *Positive for Influenza A (H1N1)pdm09* [A]
+	-	-	+	+*	Influenza A and Influenza A/H3N2 specific RNA detected. *Positive for Influenza A/H3N2* [A]
-	+	+	-	+*	Influenza B and Influenza A (H1N1)pdm09 specific RNA detected. *Positive for Influenza B and Influenza A (H1N1)pdm09* [B]
-	+	-	+	+*	Influenza B and Influenza A/H3N2 specific RNA detected. *Positive for Influenza B and Influenza A/H3N2* [C]
-	-	+	+	+*	Influenza A (H1N1)pdm09 and Influenza A/H3N2 specific RNA detected. *Positive for Influenza A(H1N1)pdm09 and Influenza A/H3N2* [B, C]
+	-	-	-	+*	Influenza A specific RNA detected. *Positive for Influenza A*
-	+	-	-	+*	Influenza B specific RNA detected. *Positive for Influenza B*

-	-	+	-	+*	Influenza A(H1N1)pdm09 specific RNA detected. *Positive for Influenza A(H1N1)pdm09* [B]
-	-	-	+	+*	Influenza A/H3N2 specific RNA detected. *Positive for Influenza A/H3N2* [C]
-	-	-	-	+	Influenza A, Influenza B, Influenza A(H1N1)pdm09 and Influenza A/H3N2 specific RNA not detected. The samples do not contain detectable amounts of influenza A/B specific RNA.
-	-	-	-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

Note: For Influenza A *M* gene (Quasar705 channel), Influenza B *HA* gene (Tex615 channel), Influenza A(H1N1)pdm09 *HA* gene (Cy5 channel) and Influenza A/H3N2 *HA* gene (FAM channel) "+" refers to amplification curve detected at CT ≤ 45 cycles. "-" refers to no amplification / no CT obtained.

* Detection of the Internal Control in the HEX channel is not required for positive results in the Quasar705/Tex615/Cy5/FAM detection channels. A high Influenza load in the sample can lead to reduced or absent Internal Control signals.

[A] Influenza A(H1N1)pdm09 and Influenza A/H3N2 strains belong to the Influenza A virus group. Therefore, A(H1N1)pdm09 and A/H3N2 positive samples generate a positive signal in the Influenza A (Quasar705) channel.

[B] Due to different detection sensitivity of Influenza A (Quasar705) and the Influenza A(H1N1)pdm09 (Cy5) assays, in rare cases weak positive samples may show a signal in the Cy5 channel but not in the Quasar705 channel.

[C] Due to different detection sensitivity of Influenza A (Quasar705) and the Influenza A/H3N2 (FAM) assays, in rare cases weak positive samples may show a signal in the FAM channel but not in the Quasar705 channel.

14.2.1 Baseline Settings for Cycler Software

After the run is completed, normalize fluorescence signals in all channels by selecting “Apply Fluorescence Drift Correction” from the Baseline Setting menu.

14.2.2 Threshold Settings for Cycler Software

Cycler	Threshold				
	Quasar705 Flu A **Channel**	**Tex615** Flu B **Channel**	**Cy5** Flu A (H1N1) pdm09 **Channel**	**FAM** Flu A/H3N2 **Channel**	**HEX** IC **Channel**
CFX96™	100 RFU	100 RFU	100 RFU	100 RFU	100 RFU
CFX Opus 96	100 RFU	100 RFU	100 RFU	100 RFU	100 RFU

14.2.3 CT Cut-Off Values for Clinical Samples

	Quasar705 Flu A **Channel**	**Tex615** Flu B **Channel**	**Cy5** Flu A (H1N1)pdm09 **Channel**	**FAM** Flu A/H3N2 **Channel**
CT Cut-Off Value	< 45 cycles	< 45 cycles	< 45 cycles	< 45 cycles

15. Performance Evaluation

The analytical performance evaluation of the LyteStar™ Influenza Typing RT-PCR Kit 1.0 was accomplished using quantified Influenza specific RNA.

15.1 Analytical Sensitivity

The analytical sensitivity (limit of detection:LoD) of the LyteStar™ Influenza Typing RT-PCR Kit 1.0 is defined as the concentration of Influenza RNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity was determined in consideration of a selected nucleic acid extraction method, by analyzing samples with known Influenza concentration.

A dilution series of the Amplirun® Total Respiratory Viral Panel (swab) was prepared by spiking into viral transport medium (VTM) and extracted with the SpinStar™ Viral Nucleic Acid Extraction Kit 1.0. Extracted Influenza RNA was analyzed with LyteStar™ Influenza Typing RT-PCR Kit 1.0. Results were analyzed by Probit analysis (Table 1, Table 2, Table 3 and Table 4).

Nucleic Acid Extraction Procedure:

SpinStar™ Viral Nucleic Acid Extraction Kit 1.0 (AstronDX Technologies)
Sample volume: 200 µl
Elution volume: 60 µl

The analytical sensitivity of the LyteStar™ Influenza Typing RT-PCR Kit 1.0 in consideration of SpinStar™ nucleic acid extraction method was determined at 0.28 copies/µl for Influenza A *M* gene target, 0.41 copies/µl for Influenza B *HA* gene target, 0.96 copies/µl for Influenza A(H1N1)pdm09 *HA* gene target and 1.61 copies/µl for Influenza A/H3N2 *HA* gene target ($p \leq 0.05$).

Table 1. PCR results used for the calculation of the analytical sensitivity of Influenza A *M* gene target for the LyteStar™ Influenza Typing RT-PCR Kit 1.0 in consideration of a particular extraction method and in combination with the CFX96™ platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
14.14	12	12	100
7.07	12	12	100
4.46	12	12	100
1.42	12	12	100
0.45	12	12	100
0.14	12	0	0
0.045	12	0	0
0.014	12	0	0
0.0045	12	0	0
0.0014	12	0	0

Table 2. PCR results used for the calculation of the analytical sensitivity of Influenza B *HA* gene target for the LyteStar™ Influenza Typing RT-PCR Kit 1.0 in consideration of a particular extraction method and in combination with the CFX96™ platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
13.17	12	12	100
6.58	12	12	100
4.15	12	12	100
1.32	12	2	16.7
0.41	12	0	0
0.13	12	4	33.3
0.04	12	1	8.3
0.013	12	0	0
0.004	12	0	0
0.001	12	0	0

Table 3. PCR results used for the calculation of the analytical sensitivity of Influenza A(H1N1)pdm09 *HA* gene target for the LyteStar™ Influenza Typing RT-PCR Kit 1.0 in consideration of a particular extraction method and in combination with the CFX96™ platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
20.0	12	12	100
10.0	12	12	100
6.3	12	12	100
2.0	12	12	100
0.63	12	2	16.7
0.20	12	0	0
0.063	12	0	0
0.020	12	0	0
0.0063	12	0	0
0.0020	12	0	0

Table 4. PCR results used for the calculation of the analytical sensitivity of Influenza A/H3N2 *HA* gene target for the LyteStar™ Influenza Typing RT-PCR Kit 1.0 in consideration of a particular extraction method and in combination with the CFX96™ platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
14.14	12	12	100
7.07	12	12	100
4.46	12	12	100
1.42	12	11	91.7
0.45	12	2	16.7
0.14	12	0	0
0.045	12	0	0
0.014	12	0	0
0.0045	12	0	0
0.0014	12	0	0

15.2 Analytical Specificity

The analytical specificity of the LyteStar™ Influenza Typing RT-PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that the applied primer/probes in LyteStar™ Influenza Typing RT-PCR Kit 1.0 specifically detect Influenza A, Influenza B, Influenza A(H1N1)pdm09 and Influenza A/H3N2.

The specificity of the LyteStar™ Influenza Typing RT-PCR Kit 1.0 was evaluated by testing genomic RNA/DNA extracted from other pathogens likely to be present in the same sample material as Influenza virus, or that cause similar symptoms to the Influenza virus (Table 5).

Table 5. Microorganisms tested to demonstrate the analytical specificity of the LyteStar™ Influenza Typing RT-PCR Kit 1.0.

LyteStar™ Influenza Typing RT-PCR Kit 1.0					
Organisms	***Flu A (Quasar 705 channel)***	***Flu B (TEX 615 channel)***	***Flu A (H1N1) pdm09 (Cy5 channel)***	***Flu A/H3N2 (FAM channel)***	***Internal Control (HEX channel)***
Human metapneumovirus	negative	negative	negative	negative	valid
Enterovirus 68	negative	negative	negative	negative	valid
Enterovirus 71	negative	negative	negative	negative	valid
Enterovirus (Coxsackie)	negative	negative	negative	negative	valid
Human respiratory syncytial virus	negative	negative	negative	negative	valid
Human rhinovirus 16	negative	negative	negative	negative	valid
Human parainfluenza virus 1	negative	negative	negative	negative	valid
Human parainfluenza virus 2	negative	negative	negative	negative	valid
Human parainfluenza virus 3	negative	negative	negative	negative	valid
Human adenovirus 1	negative	negative	negative	negative	valid
Human coronavirus OC43	negative	negative	negative	negative	valid
Human coronavirus NL63	negative	negative	negative	negative	valid
Human coronavirus HKU1	negative	negative	negative	negative	valid
Human coronavirus 229E	negative	negative	negative	negative	valid
Haemophilus influenza	negative	negative	negative	negative	valid
Legionella pneumophila subsp. *Pneumophila*	negative	negative	negative	negative	valid
Mycobacterium tuberculosis	negative	negative	negative	negative	valid
Streptococcus pneumoniae	negative	negative	negative	negative	valid
Streptococcus pyogenes	negative	negative	negative	negative	valid
Bordetella pertussis	negative	negative	negative	negative	valid
Bordetella parapertussis	negative	negative	negative	negative	valid
Mycoplasma pneumoniae	negative	negative	negative	negative	valid
Chlamydophila pneumoniae	negative	negative	negative	negative	valid
Moraxella catarrhalis	negative	negative	negative	negative	valid

The LyteStar™ Influenza Typing RT-PCR Kit 1.0 did not cross-react with any of the specified organisms.

15.3 Diagnostic Sensitivity and Specificity

The clinical performance of the LyteStar™ Influenza Typing RT-PCR Kit 1.0, in regards to diagnostic sensitivity and specificity, was evaluated through retrospective studies at various public health laboratories. The diagnostic sensitivity and specificity of the LyteStar™ Influenza Typing RT-PCR Kit 1.0 was compared to the clinical site's reference method.

In comparison to the reference test, the LyteStar™ Influenza Typing RT-PCR Kit 1.0 achieved 100% diagnostic sensitivity and 96.77% diagnostic specificity for the detection of Influenza A, Influenza B, Influenza A(H1N1)pdm09 and Influenza A/H3N2.

		Reference Method	
		Influenza Positive (n=90)	**Influenza Negative (n=31)**
LyteStar™ Influenza Typing RT-PCR Kit 1.0	**Detected**	90	1
	Not Detected	0	30
	Total	**90**	**31**
Overall Concordance		**100 %** 90 / 90 Sensitivity	**96.77 %** 30 / 31 Specificity

To calculate the diagnostic sensitivity of LyteStar™ Influenza Typing RT-PCR Kit 1.0:

$$\text{Diagnostic Sensitivity [\%]} = \frac{\text{“correct positive”}}{\text{(“correct positive” + “false positive”)}} \times 100$$

To calculate the diagnostic specificity of LyteStar™ Influenza Typing RT-PCR Kit 1.0:

$$\text{Diagnostic Specificity [\%]} = \frac{\text{“correct negative”}}{\text{(“correct negative” + “false negative”)}} \times 100$$

16. Technical Support

For customer support, please contact our Technical Support:

e-mail: **techsupport@astrondx.com**
phone: **+603 7931 6760**

17. Appendix

Explanation of Symbols

Symbol	Explanation
	*In vitro* diagnostic medical device
	Product Number
	Batch Code
	Manufacturer
	Date of Manufacture
	Contains sufficient for “n” tests/rxns
	Temperature limitation
	Version
	Use-By Date

18. Ordering Information

Products	Packing (reactions)	Order No.
LyteStar™ Influenza Typing RT-PCR Kit 1.0	96	890003
SpinStar™ Viral Nucleic Acid Extraction Kit 1.0	100	811803
MagCore® Viral Nucleic Acid Extraction Kit, High Sensitivity (200µl/400µl), CART CODE 203	96	MVN400-06

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