altona DIAGNOSTICS

Instructions for Use

RealStar®
PIV RT-PCR Kit 2.0

11/2019 EN

RealStar®

RealStar®

PIV RT-PCR Kit 2.0

For use with

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens Healthcare)

ABI Prism® 7500 SDS (Applied Biosystems)

ABI Prism® 7500 Fast SDS (Applied Biosystems)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

CFX96™ Real-Time PCR Detection System (Bio-Rad)

CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

LightCycler® 480 Instrument II (Roche)

CE

IVD

REF 262013

Σ 96

11 2019

altona Diagnostics GmbH • Mörkenstr. 12 • D-22767 Hamburg

MAN-262010-EN-S03

Content

1. Intended Use

The RealStar® PIV RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of human parainfluenza virus (PIV) specific RNA of the species 1, 2, 3 and 4 (PIV-1, PIV-2, PIV-3, PIV-4). Furthermore, the test allows the differentiation between RNA specific for the genus *Respirovirus* (PIV-1 and PIV-3) and the genus *Rubulavirus* (PIV-2 and PIV-4).

2. Kit Components

Lid Color	Component	Number of Vials	Volume [µl/Vial]
White	Water (PCR grade)	1	500
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	Positive Control PIV-1 + PIV-2	1	250
Orange	Positive Control PIV-3 + PIV-4	1	250

3. Storage

- The RealStar® PIV RT-PCR Kit 2.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit (see chapter 8.1 Sample Preparation)
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

NOTE

It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).

5. Background Information

Human parainfluenza viruses (PIV) are negative sense, single stranded RNA viruses of the family *Paramyxoviridae*. Human PIV are divided into four species belonging to two different genera: PIV-1 and PIV-3 are assigned to the genus *Respirovirus*, while PIV-2 and PIV-4 are assigned to the genus *Rubulavirus*. Two subspecies were described for PIV-4 (PIV-4a and PIV-4b) shortly after this virus was identified in 1959. Today, the existence of miscellaneous genotypes has been reported for all PIV species.

Infections with PIV are, aside *human respiratory syncytial virus* (RSV, Human respiratory syncytial virus), the second most common cause of severe lower respiratory tract illness (LRTI) in young children. Serologic surveys have shown that 90% to 100% of children aged 5 years and older have antibodies to PIV-3, and about 75% have antibodies to PIV-1 and PIV-2. Infections with parainfluenza viruses are also a significant problem in the elderly, in persons with cardiopulmonary diseases and in immunocompromised individuals. Repeated re-infections occur throughout life, but are usually manifested by a mild upper respiratory tract illness (URTI) in adults.

In general, human PIV have been associated with every kind of URTI and LRTI. The following relationships between the species and specific clinical syndromes, age of the patients as well as the outbreak season are often observed:

- PIV-1 is the major cause of acute croup in infants and young children but also causes mild upper respiratory tract infections, pharyngitis and tracheobronchitis in all age groups. In temperate climates, PIV-1 causes biennial outbreaks of croup in the fall months.
- PIV-2 is generally associated with lower infection rates than PIV-1 or PIV-3 and causes mild URTI as well as croup in children, and occasionally, LRTI. Like PIV-1, outbreaks tend to occur mostly in fall months with annual or biennial frequency.
- PIV-3 is a major cause of severe LRTI in infants and young children, often causing croup, bronchitis and pneumonia in children younger than 1 year

of age. In older children and adults, it can cause URTI or tracheobronchitis. Infections with PIV-3 can occur in any season, with peak activity during the spring and early summer months of each year.

- PIV-4 is the least common of this group and is generally associated with mild URTI.

NOTE	
i	***Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.***

6. Product Description

The RealStar® PIV RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of human parainfluenza virus (PIV) specific RNA of the species 1, 2, 3 and 4 (PIV-1, PIV-2, PIV-3, PIV-4). Furthermore, the test allows the differentiation between RNA specific for the genus *Respirovirus* (PIV-1 and PIV-3) and the genus *Rubulavirus* (PIV-2 and PIV-4).

The assay 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 labelled with fluorescent reporter and quencher dyes.

Probes specific for PIV-1 and PIV-3 are labelled with the fluorophore FAM™, whereas the probes specific for PIV-2 and PIV-4 are labelled with the fluorophore Cy®5. The probe specific for the Internal Control is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of PIV-1/3 (genus *Respirovirus*), PIV-2/4 (genus *Rubulavirus*) and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

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 RealStar® PIV RT-PCR Kit 2.0 consists of:

- Water (PCR grade)
- Master A
- Master B
- Internal Control
- Positive Control PIV-1 + PIV-2
- Positive Control PIV-3 + PIV-4

Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, PCR mediated amplification and detection of PIV-1 - 4 specific RNA and Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® PIV RT-PCR Kit 2.0 was developed and validated to be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens Healthcare)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)

- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

6.2 Sample Types

The RealStar® PIV RT-PCR Kit 2.0 has been validated for use with the following sample type:

- Human respiratory swabs collected in universal transport medium (UTM)

The RealStar® PIV RT-PCR Kit 2.0 has been validated using the AltoStar® Purification Kit 1.5 on the AltoStar® Automation System AM16 for nucleic acid extraction and purification.

If an appropriate nucleic acid extraction procedure is applied additional sample types can be used along with the RealStar® PIV RT-PCR Kit 2.0. The suitability of the nucleic acid extraction procedure as well as the use of additional sample types has to be validated by the user.

7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Clinical specimen should always be treated as 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 specimens 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) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- 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 autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Sample Preparation

Extracted RNA is the starting material for the RealStar® PIV RT-PCR Kit 2.0.

The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

The following kits and systems are generally suitable for nucleic acid extraction:

- QIAamp® Viral RNA Mini Kit (QIAGEN)
- AltoStar® Automation System AM16
- QIAsymphony® (QIAGEN)
- NucliSENS® easyMag® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

The RealStar® PIV RT-PCR Kit 2.0 has been validated using the AltoStar® Purification Kit 1.5 on the AltoStar® Automation System AM16 for nucleic acid extraction and purification.

Alternative nucleic acid extraction systems and kits might also be appropriate. However, their suitability for use with RealStar® PIV RT-PCR Kit 2.0 has to be validated by the user.

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

CAUTION

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

CAUTION

The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® PIV RT-PCR Kit 2.0 contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

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

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

- If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, add the IC 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, always and only depends 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, set up the Master Mix according to the following pipetting scheme:

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

CAUTION

If the IC (Internal Control) was added during the sample preparation procedure, at least the negative control must include the IC.

CAUTION

No matter which method/system is used for nucleic acid extraction, never add the IC directly to the specimen.

8.3 Reaction Setup

- ► Pipette 20 µl of the Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
- ► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

- ► Make sure that each Positive Control and at least one Negative Control is used per Master Mix and run.
- ► Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.
- ► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.
- ► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed programming instructions regarding the use of the RealStar® PIV RT-PCR Kit 2.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

► Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
PIV-1 and PIV-3 specific RNA	PIV-1/3	FAM™	(None)
PIV-2 and PIV 4a/b specific RNA	PIV-2/4	Cy®5	(None)
Internal Control	IC	JOE™	(None)

9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Reverse Transcription	Hold	1	-	55	20:00
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			yes	55	00:45
			-	72	00:15

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® PIV RT-PCR Kit 2.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run

A diagnostic test run is **valid**, if the following control conditions are met:

Control ID	Detection Channel		
	FAM™	Cy®5	JOE™
Positive Control PIV-1 + PIV-2	+	+	+/-*
Positive Control PIV-3 + PIV-4	+	+	+/-*
Negative Control	-	-	+

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run

A 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.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection Channel			Result Interpretation
FAM™	Cy®5	JOE™	
+	-	+*	PIV-1 and/or PIV-3 specific RNA detected.
-	+	+*	PIV-2 and/or PIV-4a/b specific RNA detected.
+	+	+*	PIV-1 and/or PIV-3 specific RNA and PIV-2 and/or PIV-4a/b specific RNA detected.
-	-	+	Neither PIV-1 nor PIV-2 nor PIV-3 nor PIV-4a nor PIV-4b specific RNA detected. The sample does not contain detectable amounts of these specific RNAs.
-	-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results either in the FAM™ detection channel or in the Cy®5 detection channel. A high PIV RNA load in the sample can lead to reduced or absent Internal Control signals.

11. Performance Evaluation

Performance evaluation of the RealStar® PIV RT-PCR Kit 2.0 was done using virus material of the following PIV strains: PIV-1: ATCC® VR-94™; PIV-2: ATCC® VR-92™; PIV-3: ATCC® VR-93™; PIV-4a: ATCC® VR-1378™; PIV-4b: ATCC® VR-1377™.

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® PIV RT-PCR Kit 2.0 is defined as the concentration (copies/ml) of PIV-1, PIV-2, PIV-3, PIV-4a or PIV-4b specific RNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of PIV species in Universal Transport Medium. PIV-1, PIV-2, PIV-3, PIV-4a and PIV-4b virus material were provided by the American Type Culture Collection (ATCC).

Each dilution was tested in 8 replicates on 3 different days (total n = 24 per dilution) using combinations of 3 RealStar® PIV RT-PCR Kit 2.0 lots, 3 AltoStar® Purification Kit 1.5 lots and 3 AltoStar® Internal Control 1.5 lots. Runs were performed using 3 different AltoStar® Automation System AM16 and CFX96™ Deep Well Real- Time PCR Detection System instruments.

Data from all runs were combined and a probit analysis was performed to determine the 95 % LoD value.

Table 1: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of PIV-1 specific RNA

Input Conc. [copies/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
3.16E+03	24	24	100
1.00E+03	24	24	100
3.16E+02	24	24	100
1.00E+02	24	22	92
3.16E+01	24	12	50
1.00E+01	24	7	29
3.16E+00	24	3	13
1.00E+00	24	1	4

Table 2: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of PIV-2 specific RNA

Input Conc. [copies/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
3.16E+03	24	24	100
1.00E+03	24	24	100
3.16E+02	24	24	100
1.00E+02	24	23	96
3.16E+01	24	17	71
1.00E+01	24	9	38
3.16E+00	24	5	21
1.00E+00	24	3	13

Table 3: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of PIV-3 specific RNA

Input Conc. [copies/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
3.16E+03	24	24	100
1.00E+03	24	24	100
3.16E+02	24	24	100
1.00E+02	24	17	71
3.16E+01	24	5	21
1.00E+01	24	1	4
3.16E+00	24	1	4
1.00E+00	24	0	0

Table 4: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of PIV-4a specific RNA

Input Conc. [copies/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
3.16E+03	24	24	100
1.00E+03	24	24	100
3.16E+02	24	23	96
1.00E+02	24	14	58
3.16E+01	24	9	38
1.00E+01	24	7	29
3.16E+00	24	0	0
1.00E+00	24	0	0

Table 5: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of PIV-4b specific RNA

Input Conc. [copies/ml]	Number of Replicates	Number of Positives	Hit Rate [%]
3.16E+03	24	24	100
1.00E+03	24	24	100
3.16E+02	24	22	92
1.00E+02	24	9	38
3.16E+01	24	3	13
1.00E+01	24	2	8
3.16E+00	24	1	4
1.00E+00	24	0	0

The analytical sensitivity of the RealStar® PIV RT-PCR Kit 2.0 was determined by Probit analysis:

- For the detection of PIV-1 specific RNA, the analytical sensitivity is 203 copies/ml [95% confidence interval (CI): 114 - 493 copies/ml]
- For the detection of PIV-2 specific RNA, the analytical sensitivity is 146 copies/ml [95% confidence interval (CI): 78 - 383 copies/ml]
- For the detection of PIV-3 specific RNA, the analytical sensitivity is 301 copies/ml [95% confidence interval (CI): 186 - 656 copies/ml]
- For the detection of PIV-4a specific RNA, the analytical sensitivity is 456 copies/ml [95% confidence interval (CI): 256 - 1,096 copies/ml]
- For the detection of PIV-4b specific RNA, the analytical sensitivity is 754 copies/ml [95% confidence interval (CI): 436 - 1,754 copies/ml]

11.2 Analytical Specificity

The analytical specificity of the RealStar® PIV RT-PCR Kit 2.0 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 all relevant PIV species will be detected.

The analytical specificity of the RealStar® PIV RT-PCR Kit 2.0 was evaluated by testing pathogens that are related to PIV and/or can cause symptoms similar to PIV.

The RealStar® PIV RT-PCR Kit 2.0 did not cross-react with any of the following pathogens:

- Human adenovirus 1
- Human adenovirus 2
- Human adenovirus 3
- Human adenovirus 4
- Human respiratory syncytial virus A
- Human respiratory syncytial virus B
- Human metapneumovirus A2
- Human metapneumovirus B2
- Influenza A virus
- Influenza B virus
- Enterovirus (Coxackievirus A3)
- Rhinovirus
- Human coronavirus
- *Bordetella pertussis*
- *Bordetella parapertussis*
- *Chlamydophila pneumoniae*
- *Mycoplasma pneumoniae*
- *Haemophilus influenzae*
- *Legionella pneumophila*
- *Moraxella catarrhalis*
- *Streptococcus pneumoniae*

Additionally PIV Species 1 to 4 were tested. The RealStar® PIV RT-PCR Kit 2.0 did not generate false positive signals in the PIV-1 and PIV-3 specific detection channel when testing PIV-2, PIV-4a or/and PIV-4b. Moreover no false positive signals in the PIV-2 and PIV-4 specific detection channel were observed when testing PIV-1 and/or PIV-3.

11.3 Precision

Precision of the RealStar® PIV RT-PCR Kit 2.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the 3 analyses.

The variability data are expressed in terms of coefficient of variation based on threshold cycle (C_t) - values. At least 6 replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

Table 6: Precision data (CV % [C_t values]) for PIV-1 high positive UTM samples

	PIV-1 High Positive Sample [CV% based on C_t value]
Intra-Assay Variability	0.41 - 2.90
Inter-Assay Variability	1.11 - 1.99
Inter-Lot Variability	1.79
Total Variability	1.56

All samples tested at 3x LoD (low positive samples) were detected positive.

Table 7: Precision data (CV % [C_t values]) for PIV-2 high positive UTM samples

	PIV-2 High Positive Sample [CV% based on C_t value]
Intra-Assay Variability	0.21 - 1.91
Inter-Assay Variability	1.38 - 2.19
Inter-Lot Variability	1.35
Total Variability	1.95

All samples tested at 3x LoD (low positive samples) were detected positive.

Table 8: Precision data (CV % [C_t values]) for PIV-3 high positive UTM samples

	PIV-3 High Positive Sample [CV% based on C_t value]
Intra-Assay Variability	0.45 - 4.04
Inter-Assay Variability	1.55 - 2.41
Inter-Lot Variability	2.80
Total Variability	2.42

All samples tested at 3x LoD (low positive samples) were detected positive.

Table 9: Precision data (CV % [C_t values]) for PIV-4a high positive UTM samples.

	PIV-4a High Positive Sample [CV% based on C_t value]
Intra-Assay Variability	0.35 - 0.77
Inter-Assay Variability	1.40 - 1.88
Inter-Lot Variability	1.07
Total Variability	1.96

All samples tested at 3x LoD (low positive samples) were detected positive.

Table 10: Precision data (CV % [C_t values]) for PIV-4b high positive UTM samples

	PIV-4b High Positive Sample [CV% based on C_t value]
Intra-Assay Variability	0.63 - 1.50
Inter-Assay Variability	2.09 - 3.07
Inter-Lot Variability	1.35
Total Variability	2.42

All samples tested at 3x LoD (low positive samples) were detected positive.

Table 11: Precision data (CV % [C_t values]) for the detection of the Internal Control in PIV negative UTM samples

	Internal Control
Intra-Assay Variability	0.33 - 0.94
Inter-Assay Variability	0.82 - 2.07
Inter-Lot Variability	0.47
Total Variability	1.64

11.4 Diagnostic Evaluation

The RealStar® PIV RT-PCR Kit 2.0 was evaluated in a comparative study with the CE marked RIDA® GENE Parainfluenza (r-biopharm) Kit.

Retrospectively, 80 respiratory swab samples were tested in parallel using the RIDA® GENE Parainfluenza (r-biopharm) Kit in combination with the MagNA PURE 96 DNA and Viral NA Small Volume Kit (Roche) and the MagNA Pure 96 System (Roche) and the RealStar® PIV RT-PCR Kit 2.0 in combination with the AltoStar® Purification Kit 1.5 and the AltoStar® Internal Control 1.5 on the AltoStar® Automation System AM16 and the CFX96™ Deep Well Real-Time PCR Detection System. For the qualitative analysis all samples with an invalid result for one or both assays were excluded. Results for the remaining 72 samples are shown in Table 12.

Table 12: Results of the evaluation of the diagnostic sensitivity and specificity of the RealStar® PIV RT-PCR Kit 2.0 in respiratory swabs

		RIDA® GENE Parainfluenza (r-biopharm)	
		POSITIVE	NEGATIVE
RealStar® PIV RT-PCR Kit 2.0	POSITIVE	32	1
	NEGATIVE	0	39

The diagnostic sensitivity and the diagnostic specificity of the RealStar® PIV RT-PCR Kit 2.0 compared to the RIDA® GENE Parainfluenza (r-biopharm) kit was 100 % and 97.5 %, respectively.

12. Limitations

- Strict compliance with the Instructions for Use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of RT-PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- Potential mutations within the target regions of the PIV genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the RealStar® PIV RT-PCR Kit 2.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH ISO EN 13485-certified Quality Management System, each lot of RealStar® PIV RT-PCR Kit 2.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For customer support, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Literature

Versalovic, James, Carroll, Karen C.,Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); AltoStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); SmartCycler® (Cepheid); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, MinElute®, QIAsymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The RealStar® PIV RT-PCR Kit 2.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

17. Explanation of Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Catalogue number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade item number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note
	Version

Notes:

always a drop ahead.

altona Diagnostics GmbH
Mörkenstr. 12
22767 Hamburg, Germany

phone +49 40 548 0676 0
fax +49 40 548 0676 10
e-mail info@altona-diagnostics.com

www.altona-diagnostics.com