ViroReal® Kit Parainfluenzavirus

Manual

CE

IVD **For *in vitro* diagnostic use**

REF **DHUV01653**

Σ **50 reactions**

ingenetix GmbH
Arsenalstraße 11
1030 Vienna, Austria
T +43(0)1 36 1980 198
F +43(0)1 36 1980 199
office@ingenetix.com
www.ingenetix.com

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Explanation of symbols

	Batch code		Use by
	Catalogue number		Manufactured by
	Contains sufficient for <n> tests		Store at
	This product fulfils the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices		For *in vitro* diagnostic use
	Corrosion, GHS05		Exclamation mark, GHS07

1. Intended use

ViroReal® Kit Parainfluenzavirus is an *in vitro* diagnostic test, based on one-step reverse transcription real-time PCR (RT-PCR), for the detection of parainfluenzavirus 1, 2, 3 and 4 strains (HPIV-1, HPIV-2, HPIV-3, HPIV-4).

2. Product description

ViroReal® Kit Parainfluenzavirus detects RNA of the hemagglutinin-neuraminidase glycoprotein gene (HN gene) of human parainfluenzavirus types 1, 2, 3 and 4.
This test allows the rapid and sensitive detection of RNA of parainfluenzavirus from samples purified from the respiratory tract (e.g. with the QIAamp Viral RNA Mini Kit, Qiagen).

A probe-specific amplification-curve in the FAM channel indicates the amplification of parainfluenzavirus type 1 and 3 (HPIV-1 and HPIV-3) specific RNA, while a probe-specific amplification-curve in the VIC/HEX channel indicates the amplification of parainfluenzavirus type 2 and 4 (HPIV-2 and HPIV-4) specific RNA. An internal RNA positive control (RNA IPC) is detected in Cy5 channel and is used as RNA extraction as well as RT-PCR inhibition control. The target for the RNA IPC is extracted with the sample.

This test has been validated with the Applied Biosystems® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) and tested with a LightCycler® 480 Instrument II (Roche) and Mx3005P® QPCR System (Agilent), but is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM, VIC/HEX and Cy5 channel.

The test is based on one-step reverse transcription real-time PCR (RT-PCR). A specific RNA sequence of the pathogen genome is transcribed into cDNA and amplified in a one-step PCR. The generated PCR-product is detected by an oligonucleotide-probe labelled with a fluorescent dye. This technology allows a sequence-specific detection of PCR amplificates.

Ingenetix ViroReal®, BactoReal® and ParoReal Kits are optimized to run under the same thermal cycling conditions. RNA and DNA can be analysed in one run.

3. Pathogen information

Human parainfluenzaviruses (HPIV) are enveloped, negative single-stranded RNA viruses (ss(-)RNA) of the family *Paramyxoviridae*. At present four serotypes are known (types 1, 2, 3, 4). Parainfluenzavirus type 1 and 3 belong to the genus *Respirovirus*, while parainfluenzavirus type 2 and 4 belong to the genus *Rubulavirus.*
Human parainfluenzavirus type 1 (HPIV-1) is the major cause of acute croup, but can also cause respiratory tract infections at all ages. Human parainfluenzavirus type 2 (HPIV-2) can also cause croup as well as upper and lower respiratory tract illnesses. Human parainfluenzavirus type 3 (HPIV-3) causes bronchiolitis and pneumonia. Human parainfluenzavirus type 4 with the subtypes 4a and 4b is less likely to cause servere disease.

4. Contents of the kit, stability and storage

Labelling	Content	Amount	Storage
Parainfluenzavirus 1-4 Assay Mix (green cap)	Primer and probe (FAM and VIC/HEX) for detection of HPIV-1, HPIV-2, HPIV-3 and HPIV-4	1 x 50 µl	-15 °C to -25 °C
RNA IPC-3 Assay Mix (yellow cap)	Primer and probe (Cy5) for RNA IPC detection	1 x 50 µl	-15 °C to -25 °C
RNA IPC Target (orange cap)	Target for RNA IPC (internal RNA positive control system)	1 x 100 µl	-15 °C to -25 °C
Parainfluenzavirus 1, 2 Positive Control (red cap)	RNA positive control for HPIV-1 and HPIV-2	1 x 15 µl	-15 °C to -25 °C
RNA Reaction Mix (white cap)	4 x Reaction Mix	1 x 250 µl	-15 °C to -25 °C
Nuclease-free water (blue cap)	Nuclease-free water	1 x 1000 µl	-15 °C to -25 °C

The components of ViroReal® Kit Parainfluenzavirus are stable until the expiry date stated on the label.

5. Additionally required materials and devices

- Reagents and devices for RNA-extraction
- Nuclease-free water for dilution of RNA IPC Target and positive control
- Disposable powder-free gloves
- Pipettes (adjustable)
- Sterile pipette tips with filters
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Real-time PCR instrument which is able to detect and differentiate fluorescence in FAM, VIC/HEX and Cy5 channel
- Optical 96 well reaction plates or optical reaction tubes

6. Precautions and safety information

- For *in vitro* diagnostic use. The use of this kit is limited to persons instructed in the procedures of real-time PCR and *in vitro* diagnostics.
- Clean benches and devices periodically.
- Use sterile filter pipette tips.
- Specimens should be handled as if infectious in accordance with safe laboratory procedures. Wear protective disposable powder-free gloves when handling kit reagents and specimens.
- Use separated working areas for specimen preparation, reagent preparation and amplification. Supplies and equipment must be dedicated to each of these separate working areas and ensure workflow in the laboratory from pre- to post-PCR.
- Be careful when handling samples and positive control to avoid cross contamination. Change gloves after handling of samples or positive control. Store positive or potentially positive material separately from reagents
- Prevent contamination of work equipment and reagents with DNA/RNA, nuclease or amplification products by good laboratory practice.
- Quality of RNA has a profound impact on the test performance. Ensure that the used RNA extraction system is compatible with reverse transcription real-time PCR technology.
- Always include a negative control per PCR-run (nuclease-free water instead of sample).
- For a valid interpretation of results, a negative control should be included during RNA-extraction (e.g. extraction of water instead of sample material), in order to exclude false-positive results due to contamination with virus RNA during extraction.
- Please note the expiry date of the kit.
- Do not interchange or mix reagents from kits with different lot numbers.

- Repeated thawing and freezing of kit components should be avoided. Protect kit components from light. **Caution:** Positive Control and RNA IPC Target are stored in RNA stabilizer which contains Guanidinium thiocyanate/Triton X-100 (see MSDS, www.ingenetix.com).
- Use established laboratory practices according to your local safety regulations for discarding specimens, reagents and waste.

7. Limitations

- Optimal performance of this test requires appropriate specimen collection, transport and storage, as well as an appropriate RNA extraction procedure. RNA extraction and parainfluenzavirus detection have been validated for nasopharyngeal swabs with this kit. Test performance with other specimen types has not yet been assessed.
- A negative test result does not exclude the possibility of parainfluenzavirus infection, because test results may be affected by improper specimen collection, technical error, specimen mix-up or viral quantities below the assay sensitivity. The presence of PCR inhibitors may lead to invalid results.
- For this kit highly specific primers and probes have been selected. However, false-negative or less sensitive results might be obtained due to sequence heterogeneity within the target region of not yet described clinical subtypes.
- Results should be interpreted in consideration of clinical and laboratory findings.

8. Preparation of samples

Extract samples with a RNA extraction system compatible with reverse transcription real-time PCR technology. An extraction negative control should be included during RNA-extraction (e.g. extraction of water instead of sample material).
The **RNA IPC Target** has to be added during extraction. The RNA IPC is used as a control of RNA extraction, identifies possible PCR inhibition and confirms the integrity of kit reagents.
Caution: The RNA IPC Target must not be added directly to the sample material but has to be pipetted to the lysis buffer.
→ For an elution volume of 50-100 µl: Per sample, spike 1 µl RNA IPC Target into lysis buffer.
→ For an elution volume of >100 µl or when using an automated extraction system: Per sample, spike 2 µl RNA IPC Target into lysis buffer.

Use RNA immediately after extraction (always store on ice) and store at -80°C as soon as possible.

9. Preparation of real-time PCR

- Include one negative control (water), one positive control and one extraction negative control per PCR run.
- It is recommended to analyse samples in duplicates, which increases the probability of pathogen detection and facilitates interpretation of results.
- Thaw RNA samples on ice.
- Thaw RNA Reaction Mix on ice. Invert the RNA Reaction Mix 2 to 3 times to ensure homogeneity of solution. Avoid warming to room temperature. Thaw all other kit components thoroughly at room temperature. When thawed, mix components, centrifuge briefly and keep on ice.
- Prepare master mix on ice.
- **Parainfluenzavirus Positive Control** is an *in vitro* synthesized fragment of HPIV-1 and HPIV-2 RNA in RNA-stabilizer. Before use, freshly dilute positive control 1:500 with nuclease-free water.
  → Use 1 µl of freshly 1:500 diluted Parainfluenzavirus Positive Control + 9 µl nuclease-free water.
  Caution: The use of more than 1 µl diluted (1:500) positive control per reaction causes inhibition of the RT-PCR reaction. Pipette positive control at last.

9.1. Pipetting scheme

		Per sample
Preparation of Master Mix (mix well)	Nuclease-free Water*	3.0 µl
	RNA Reaction Mix	5.0 µl
	Parainfluenzavirus 1-4 Assay Mix	1.0 µl
	RNA IPC-3 Assay Mix	1.0 µl
	Total volume Master Mix	**10.0 µl**
Preparation of PCR	Master Mix	10.0 µl
	RNA-Sample*	10.0 µl
	Total volume	**20.0 µl**

*1-10 µl of the sample can be used. When using < 10 µl sample, the volume of water has to be adjusted accordingly.

→ **If RNA IPC Target was not added during extraction:** Freshly dilute the RNA IPC Target 1:500 with nuclease-free water and add 1 µl per sample directly to the master mix.
Caution: The use of more than 1 µl diluted (1:500) RNA IPC Target per reaction causes inhibition of the RT-PCR reaction.

9.2. Programming of temperature profile

Please find further information on programming the real-time PCR instrument in the respective operator's manual. Take into consideration that some PCR-platforms have to be calibrated with the corresponding dye before performing a multiplex-PCR.

Select detection channel: FAM-TAMRA, 530 nm (for HPIV-1 and HPIV-3)
VIC/HEX, 554 nm (for HPIV-2 and HPIV-4)
Cy5-NONE, 667 nm (for RNA IPC-3)

Select reference dye (passive reference): ROX

Sample Volume: 20 µl

Temperature Profile:

Program 1	Program 2	Program 3
Cycles: 1 Analysis: None	Cycles: 1 Analysis: None	Cycles: 45 Analysis: Quantification Acquisition at 60°
50°C 15 min	95°C 20 sec	95°C 5 sec 60°C 1 min

<u>For ABI PRISM® 7500:</u>
Ramp speed: "Standard"

<u>For LightCycler® 480 instrument:</u>
Detection format: 3 Color Hydrolysis Probe

Note: These parameters are valid for all ingenetix ViroReal®, BactoReal®, MycoReal and ParoReal kits.

10. Interpretation of PCR-data

For analysis of PCR results select fluorescence display options 530 nm (FAM channel) for the HPIV-1 and HPIV-3 target, 554 nm (VIC/HEX channel) for the HPIV-2 and HPIV-4 target, and 667 nm (Cy5 channel) for the RNA IPC target. Samples with positive Ct or Cp-values are considered positive. Please additionally check the amplification-curves manually. Samples should be inspected both in logarithmic and linear scale view and compared with the negative control.

	FAM channel HPIV-1, 3 target	VIC/HEX channel HPIV-2, 4 target	Cy5 channel RNA IPC target	Interpretation
Negative control	Negative	Negative	Negative / Positive[1]	Valid
Positive control (1:500)	Positive	Positive	Negative / Positive[1]	Valid
Extraction negative control	Negative	Negative	Positive	Valid
Sample	Positive	Positive	Positive / Negative[2]	Positive
Sample	Positive	Negative	Positive / Negative[2]	Positive
Sample	Negative	Positive	Positive / Negative[2]	Positive
Sample	Negative	Negative	Positive	Negative[3]
Sample	Negative	Negative	Negative	Invalid

[1]Only positive if the RNA IPC Target was added at a 1:500 dilution directly to the master mix
[2]High virus load in the sample can lead to reduced or absent signal of RNA IPC
[3]The positive signal of the RNA IPC excludes a possible PCR inhibition. However, IPC Ct-values should show comparable results. A shift of Ct-values can indicate a partial inhibition of PCR.

In case of invalid data, analysis has to be repeated with the remaining or newly extracted RNA sample (see 11. Troubleshooting).

11. Troubleshooting

11.1. No signal in FAM channel, VIC/HEX channel and Cy5 channel with controls and sample:

- Incorrect programming of the temperature profile or detection channels of the real-time PCR instrument.
  → Compare temperature profile and programming of detection channels with the protocol.
- Incorrect configuration of PCR reaction.
  → Check your work steps (see pipetting scheme) and repeat PCR, if necessary.
- RNA might be degraded.
- RNA of the positive control was not freshly diluted 1:500.
  → Prepare a fresh 1:500 dilution of the positive control and repeat PCR.

11.2. Valid results for controls, but no signal in FAM channel, VIC/HEX channel and Cy5 channel with sample:

- Incorrect programming of detection channels with the sample.
  → Compare programming of detection channels with protocol.
- RNA might be degraded.
- If the RNA IPC Target was added during extraction:
  - PCR reaction has been inhibited.
  - RNA extraction has failed.
  - The undiluted RNA IPC Target has not been added to lysis buffer of sample.
  - The extracted sample has not been added to PCR reaction.

  → No interpretation can be made. Make sure you use a recommended method for RNA isolation and stick closely to the manufacturer's instructions. Check your work steps.

11.3. Signal in FAM channel and/or VIC/HEX channel in negative control:

- A contamination occurred during preparation of PCR.
  → Repeat PCR with new reagents in replicates.
  → Strictly pipette positive control at last.
  → Make sure that work space and instruments are cleaned at regular intervals.

11.4. Signal in FAM channel and/or VIC/HEX channel in extraction negative control:

- A contamination occurred during extraction.
  → Repeat extraction and PCR using new reagents.
  → Make sure that work space and instruments are cleaned at regular intervals.

12. Specifications and performance evaluation

12.1. Kit performance on different real-time PCR instruments

Performance of ViroReal® Kit Parainfluenzavirus with an Applied Biosystems® 7500 Fast Real-time PCR System (Thermo Fisher Scientific) is shown in Figures 1, 2.

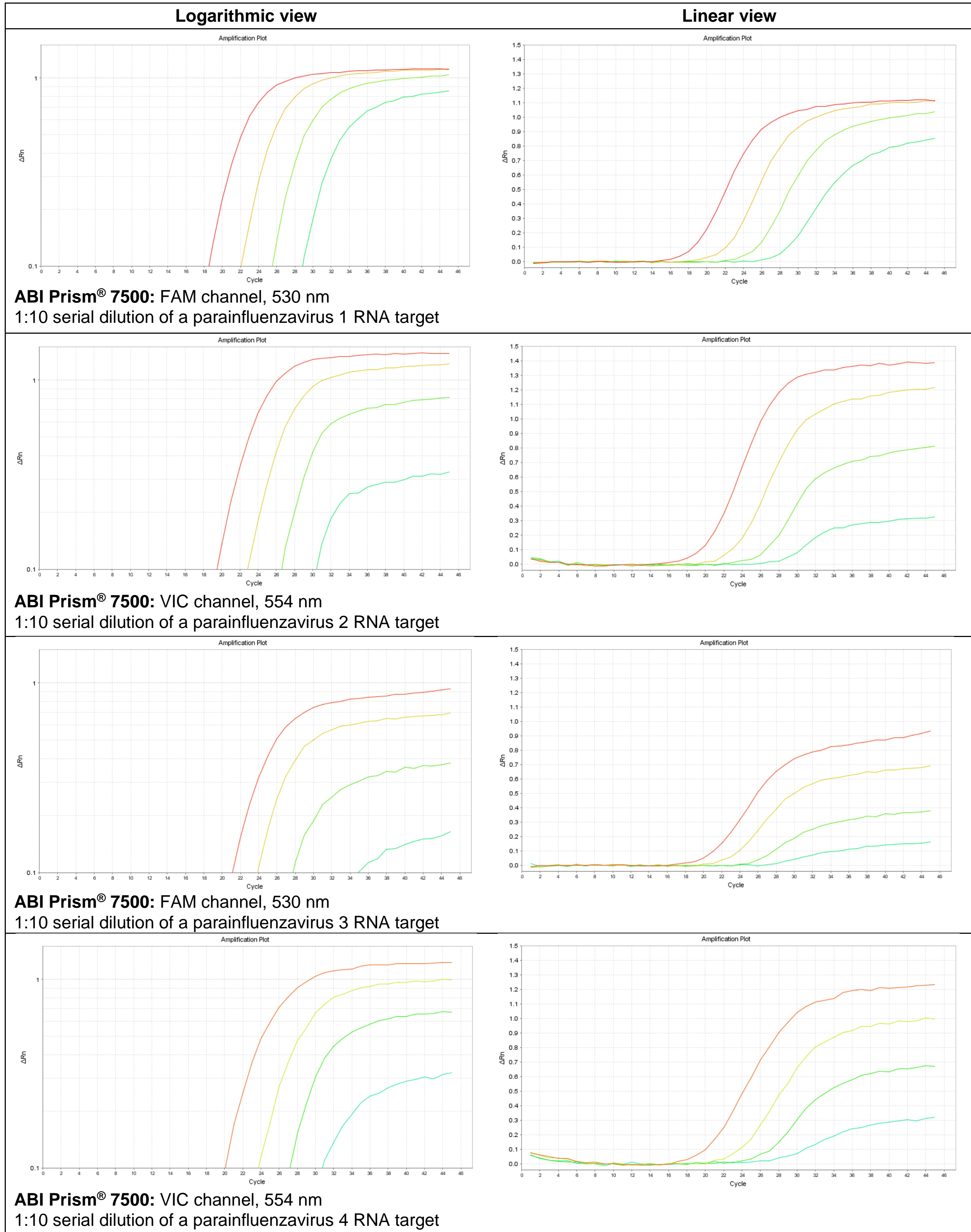

Figure 1 Performance of ViroReal® Kit Parainfluenzavirus

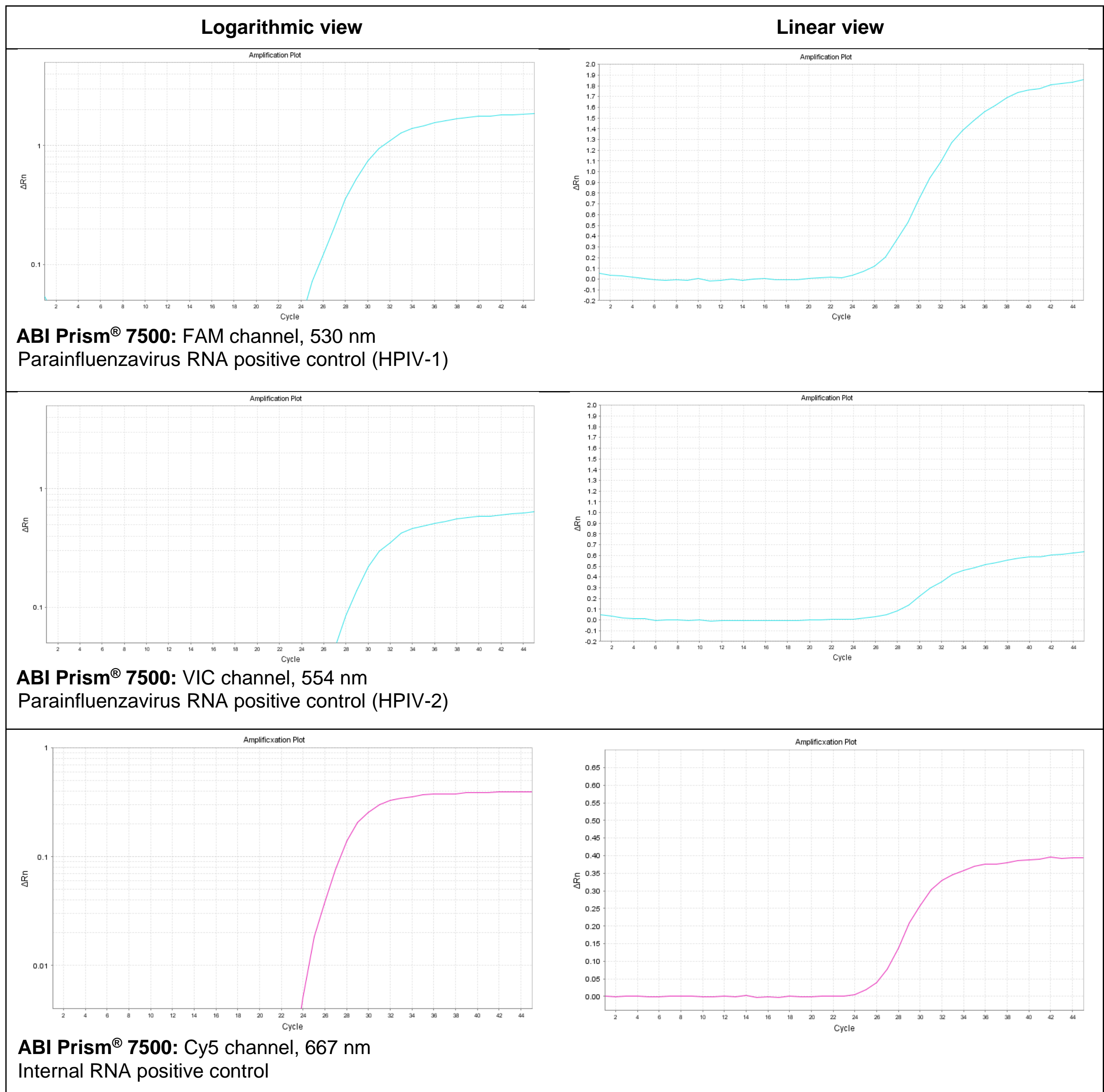

Figure 2 Performance of ViroReal® Kit Parainfluenzavirus

12.2. Limit of detection and linearity

ViroReal® Kit Parainfluenzavirus was tested with a 10-fold dilution series of a synthetic RNA representing fragments of parainfluenzavirus RNA. At least 10 target copies/reaction of HPIV-1 and at least 100 target copies/reaction of HPIV-2, HPIV-3 and HPIV-4 could be detected.

The **limit of detection** (LoD95 = smallest number of copies of target RNA which can be detected in 95% of cases) is 47 target copies/reaction for HPIV-1, 335 target copies/reaction for HPIV-2, 294 target copies/reaction for HPIV-3 and 346 target copies/reaction for HPIV-4.

The assay shows **linearity** over the range of 100 to 10,000,000 target copies/reaction with a slope of -3.5 ± 0.02 and a R_2 of 0.99 for HPIV-1. For the other serotypes, it shows linearity over the range of 1000 to 10,000,000 target copies/reaction with a slope of -3.2 ± 0.1 and $R_2 > 0.99$ for HPIV-2, with a slope of -3.9 ± 0.1 and $R_2 > 0.99$ for HPIV-3 and with a slope of -3.8 ± 0.1.and $R_2 > 0.99$ for HPIV-4 as shown in Figure 3.

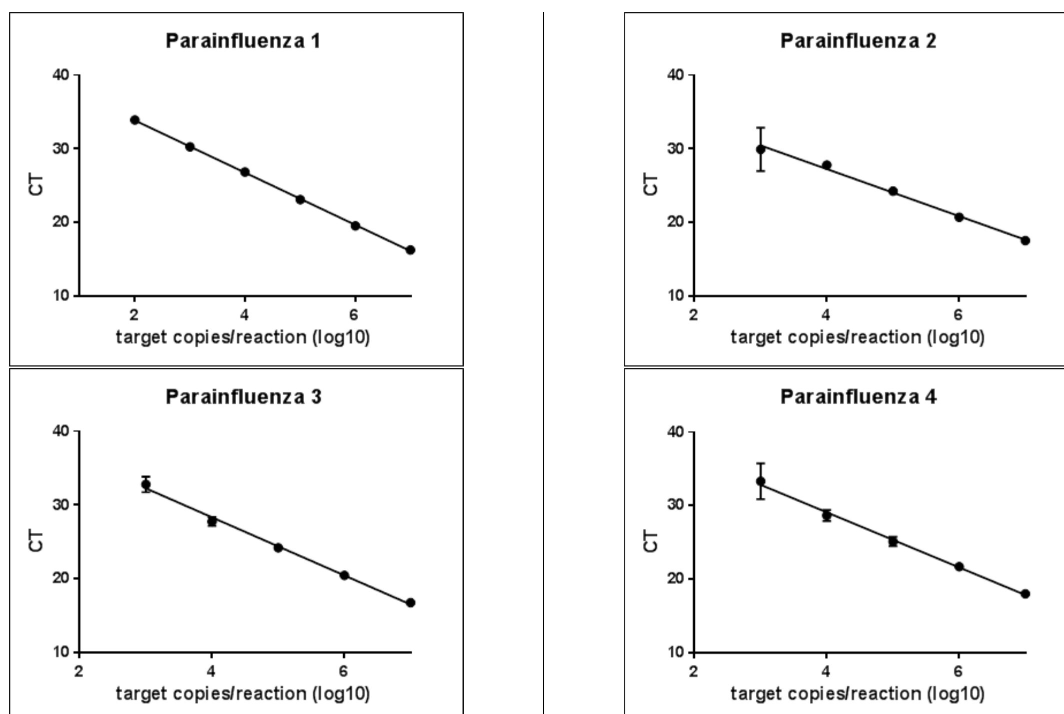

Figure 3: Ten-fold dilution series of parainfluenzavirus RNA standards plotted against CT.

12.3. Analytical specificity

The selection of highly specific primers and probes ensures analytical specificity. Primers and probes have been checked for possible homologies to currently published sequences by sequence comparison analyses. This validates the detection of so far known human parainfluenzavirus strains type 1-4.

For qualitative performance evaluation, RNA of 3 parainfluenzavirus isolates from cell culture lysates was tested with ViroReal® Kit Parainfluenzavirus. All isolates were positive for HPIV-2 and HPIV-3.

Analytical specificity has been further evaluated by testing genomic DNA or RNA of viruses (adenovirus, enterovirus, metapneumovirus, respiratory syncytial virus, influenza A, influenza B) and of bacteria (*Bordetella pertussis, Bordetella parapertussis, Streptoccocus pneumoniae, Mycoplasma pneumoniae, Neisseria meningitidis*, *Haemophilus influenzae*). No cross-reactions were observed.

12.4. Diagnostic evaluation

Diagnostic evaluation was performed with 27 parainfluenza-positive isolates (10 isolates from nasopharyngeal aspirates, 17 isolates from cell culture and ring trial samples). From these, 9 isolated were of serotype 1, 7 isolates of serotype 2, 10 isolates of serotype 3 and one isolate of serotype 4. No signal was obtained with 15 parainfluenza-negative samples.

13. References

Henrickson KJ. 2003. Parainfluenzaviruses. Clin Microbiol Rev. 16:242-264.