10. Sample Data - Typical Results

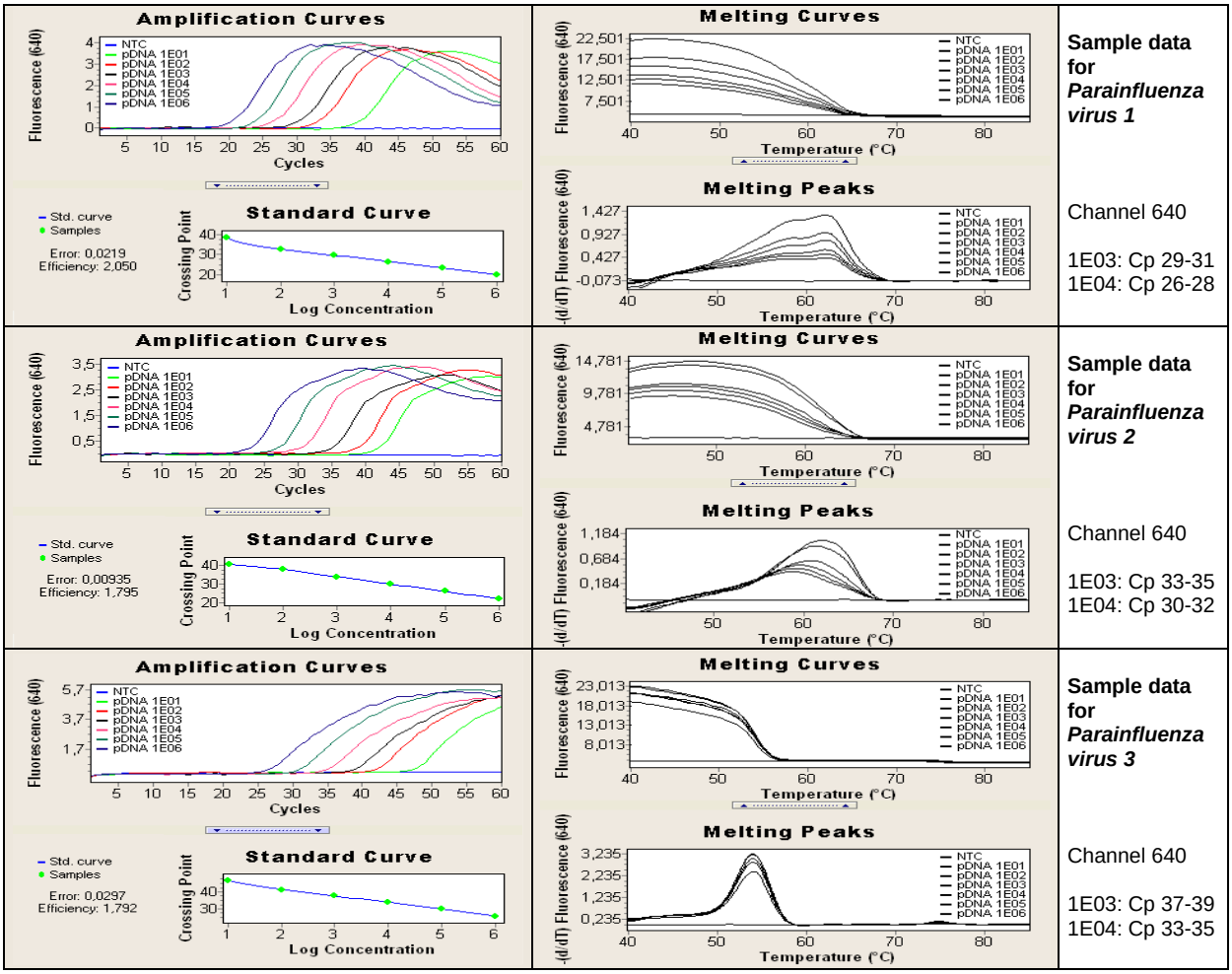

Fig.1. LightCycler® 2.0 Sample data for the *Parainfluenza virus 1, 2, 3* detection system.

Left panel Quantification (Second Derivative Maximum) with calibration curve.
Right panel Melting curves for the target (not relevant for detection, shape varies with concentration).

11. Interpretation of Data

Sample	NTC	Result
No amplification	negative	Negative (not detectable)
Amplification signal	negative	Positive for Parainfluenza virus
Amplification signal	positive	Contamination, repeat experiment

Tab. 3. Typical analysis results (LightCycler® 2.0 Instrument, Roche Master: Fast Start)

12. Version History

Notes in red mark events require to change procedures

V061101 Release version
V130813 MSDS included, Editorial changes

Roche SAP order n° 05997801001

Notice to Purchaser

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These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany.
LightCycler® hybridization probes produced under license from Roche Diagnostics.

For life science research use only. Not for use in diagnostic procedures. For *in vitro* use only.

LightMix® Kit *Parainfluenza virus 1, 2, 3*

Cat.-No. 40-0223-16

Kit with reagents for detection of *Parainfluenza virus 1, 2, 3 c*DNA using LightCycler® 1 / 2.0 Systems.

Lyophilized mix of primers and probes for a total of 96 reactions with a final volume of 20 µl each. **Store protected from light at room temperature (18-25°C), do N OT freeze!**

1. Introduction

Parainfluenza virus (PIV) 1, 2, 3 are RNA viruses, where subtypes 1 and 3 belong to the genus paramyxovirus and subtype 2 to the genus rubullavirus. Haemagglutinin and neuraminidase are present. Parainfluenza virus produce disease throughout the year, croup, or laryngotracheobronchitis is the commonest clinical manifestations. PIV-1 and 2 are particularly prone to produce croup whilst PIV-3 is prone to produce bronchitis and pneumonia. Infections with Parainfluenza virus are primarily childhood diseases. The virus enters the host through the inhalation of infected droplet nuclei.

This kit provides a fast, easy and accurate system to identify these targets in a nucleic acid extract. The kit is tested with the 'LightCycler® FastStart DNA Master HybProbe' and 'Master^{PLUS} HybProbe' using LightCycler® 1.x / 2.0 Instruments. A 1-step RT PCR procedure was not tested.

2. Description

A 317 bp (*PIV-1*), 204 bp (*PIV-2*), or 103 bp (*PIV-3*) fragment of the virus genome is amplified with specific primers and detected with probes labeled with LightCycler® Red 640 (detection channel 640).

The supplied standard rows allow to determine the linear range of the reaction and to estimate the quantity of the target sequence in unknown samples.

For use in LightCycler® 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler® 1.x Instruments to software version 4.1.

3. Set Contents

6	Vials with blue caps containing premixed lyophilized primers and probes for 16 reactions PIV-1
6	Vials with blue caps containing premixed lyophilized primers and probes for 16 reactions PIV-2
6	Vials with blue caps containing premixed lyophilized primers and probes for 16 reactions PIV-3
3	Row with 6 lyophilized standards *PIV 1, 2, 3* DNA from 10^1 to 10^6 target equivalents per reaction
3	Sealing foilsfor the standard rows

4. Additional Reagents and items required

	Roche Diagnostics
LightCycler® FastStart DNA Master^{PLUS} HybProbe	Cat.-No. 03 515 575 001
or LightCycler® FastStart DNA Master HybProbe	Cat.-No. 03 003 248 001
High Pure Viral Nucleic Acid Kit	Cat.-No. 11 858 874 001
Transcriptor First Strand cDNA Synthesis Kit	Cat.-No. 04 379 012 001

5. Material Safety Data

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC and 1999/45/EC any products which not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

Product is not hazardous, not toxic, not IATA-restricted. Product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

6. Programming

The protocol consists of four program steps

- 1: Denaturation: samples denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:	Denaturation	Cycling			Melting				Cooling
Parameter									
Analysis Mode	None	Quantification mode			Melting Curves mode				None
Cycles	1	60			1				1
Target [℃]	95	95	62	72	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:05	00:00:15	00:00:05	00:00:20	00:00:30	00:00:00	00:00:30
Ramp Rate [℃/s]	20	20	20	20	20	20	20	0.2	20
Sec Target [℃]	-	-	55	-	-	-	-	-	-
Step Size [℃]	-	-	0.5	-	-	-	-	-	-
Step Delay (Cycles)	-	-	1	-	-	-	-	-	-
Acquisition Mode	None	None	Single	None	None	None	None	Cont	None

(Melting not relevant for detection)

Note: Universal step-down cycling program. No changes in reagents made. The former cycling program yields equivalent results.
60 cycles instead of 50 cycles (standard universal program) are necessary to detect PIV3 DNA in low amounts (10 copies).

7. Data analysis

Perform data analysis, as described in the LightCycler® operator's manual. We recommend using the Second Derivative Maximum method (Automated (F" max)). The cycle number of the Crossing Point (Cp) of each sample is calculated automatically. The Fit Points method is more-error prone due to user's influences.

View *Parainfluenza virus 1, 2, 3* data in channel 640, Quantification mode. The negative control (NTC) must show no signal.

The provided standard rows of cloned and purified DNA with concentrations in the range from 10^6 copies/rxn to 10^1 copies/rxn of *Parainfluenza virus 1, 2, 3* should have Cp values between cycles 18 and 37 for *Parainfluenza virus 1*, Cp values between cycles 20 and 39 for *Parainfluenza virus 2* and Cp values between cycles 25 and 47 for *Parainfluenza virus 3* (Cp values calculated with Second Derivative Maximum method).

8. Product Characteristics

PCR results are obtained within 1 hour.

Sensitivity
These reagents detect 10 copies of *Parainfluenza virus 1, 2, 3* DNA (in an exemplary system, using cloned targets as reference).

Measuring range
The linear measuring range of the assay is 10^2 to 10^6 copies of *Parainfluenza virus 1, 2, 3* DNA.

Storage and Stability
- Lyophilized reagents are stable for at least 6 months after shipment when stored protected from light at room temperature (18-25℃). See expiry date o n the product label.
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 10 days when stored protected from light and refrigerated (4℃).
- Dissolved reagents can be long-term stored frozen at -20℃. Avoid multiple thaw-freeze cycles.

9. Experimental Protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 Instruments.
Start programming before preparing the solutions. See the operator's manual for details.

Sample material: Use aqueous nucleic acid preparations (e.g. Roche Diagnostics 'High Pure Viral Nucleic Acid Kit' combined with Roche Diagnostics 'Transcriptor First Strand cDNA Synthesis Kit').

Negative control: Always run at least one negative control - replace the template DNA with water.

Positive control: Run a positive control - replace the template DNA with the provided control DNA.
For quantification always use a fresh solution prepared from the provided standard row (single use only).

(A) Preparation of parameter-specific reagents (16 reactions):

One reagent vial with a **blue** cap contains all primers and probes to run 16 reactions.

Add 66 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

►Use 4 µl **reagent** for a 20 µl PCR reaction.

This solution is stable for three days or longer if stored refrigerated at 4°C. Avoid prolonged exposu re to light.

(B) Preparation of the standard row (quantification)

The target DNA is provided in 6 different quantities to yield from 10^1 to10^6 target molecules in 5 µl once resolved. Start with the lowest concentrated standard (first tube from the extended lip). Use the pipette tip to punch a hole through the sealing foil. **Add 40 µl** PCR-grade water to each vial of the row. Mix the target DNA by pipetting the solution up and down 10 times.

►Use 5 µl **standard** for a 20 µl PCR reaction

This standard solution is not long-term stable and will loose sensitivity under prolonged storage. Use only fresh prepared solutions as quantification references. Older solutions can be used as a qualitative standard (positive control).
After adding the target DNA to the LightCycler® reaction mix use the provided sealing foil to close the vials in order to avoid changes in the concentration due to evaporation.
Please note that opening of these vials may cause contaminations of the work-space (aerosol).

(C) Preparation of the LightCycler® reaction mix

In a reaction tube cooled below 4°C, prepare the re action mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

For use with the Roche FastStart^{PLUS} kit		**For use with the Roche FastStart kit**
Single reaction	**Component**	**Single reaction**
7.0 µl	water, PCR-grade (colorless cap, provided with the Roche FastStart or FastStart^{PLUS} kit)	7.4 µl
--	Mg^{2+} solution 25 mM (blue cap, provided with the Roche FastStart kit)	1.6 µl
4.0 µl	**reagent** mix (parameter specific reagents containing primers and probes, see **A**)	4.0 µl
4.0 µl	FastStart mix (vial 1 (red cap), combined from vials 1a and 1b, see Roche manual)	2.0 µl
15.0 µl	**Volume of reaction mix**	**15.0 µl**

Mix gently, spin down and transfer 15 µl each of the reaction mix to a LightCycler® capillary.
Add 5 µl of sample or standard (standard dilutions of control target, see instruction **B**) to each capillary to give a final reaction volume of **20 µl.**

Start run.