Application Note

A Novel Approach for Identification of 16 Respiratory Viral Targets

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Introduction

Viral respiratory tract infections are associated with various types of virus, including adenovirus, coronavirus, human rhinovirus, influenza A, influenza B, metapneumovirus, parainfluenza and respiratory syncytial virus. To efficiently identify all these viruses requires the establishment of an accurate and reliable method that is rapid, sensitive and affordable.

Current non-molecular methods, such as viral culture or immunofluorescence (direct fluorescence antibody, DFA), are insufficiently sensitive, time consuming and labor intensive (1,2). Consequently, molecular methods are used and a well-accepted approach is an automated electrophoresis system to identify differences in amplicon size. Recently, multiplex RT-PCR assays have been developed specifically to detect respiratory viruses, but the limited availability of required equipment renders them difficult to standardize.

The method described herein uses a two-tube multiplex reverse-transcription PCR assay followed by amplicon-size separation using the QIAxcel® Advanced System (3). It is fast, highly automated, and based on reliable, affordable and readily available equipment and reagents. As such, this method may make an important contribution to routine virus identification.

Materials and Methods

Samples were collected and total RNA/DNA was extracted using the QIAamp® Viral RNA Mini Kit. A two-tube multiplex reverse-transcription PCR assay (two-tube assay) was used to detect 16 respiratory viruses based on their amplicon size differences. ▷

Test A was set up for simultaneous detection of nine respiratory viruses:

- Influenza A virus (FluA)
- Influenza B virus (FluB)
- Seasonal influenza A virus subtypes H1N1 (sH1N1)
- Parainfluenza virus type 1 (PIV1)
- Human rhinovirus (HRV)
- Coronavirus subtypes OC43 (CoV OC43)
- Coronavirus subtypes 229E (CoV 229E)
- Coronavirus subtypes HKU1 (CoV HKU1)
- Adenovirus (Adv)

In Test B, seven respiratory viruses were detected:

- Parainfluenza virus type 2 (PIV2)
- Parainfluenza virus type 3 (PIV3)
- Respiratory syncytial virus A (RSVA)
- Respiratory syncytial virus B (RSVB)
- Coronavirus subtypes NL63 (CoV NL63)
- Human metapneumovirus (HMPV)
- Human bocavirus (HBoV)

The QIAGEN® OneStep RT-PCR Kit was used for the amplification and PCR was performed as previously described (3). All PCR tubes (or 96-well reaction plate) can be loaded directly onto the QIAxcel Advanced electrophoresis system without post-PCR operation. For interpretation during analysis, the QIAxcel DNA High Resolution Kit in combination with the QX DNA Size Marker 25-500 bp and the QX Alignment Marker 15 bp/600 bp were used.

Results and Discussion

Test A allowed identification of nine respiratory viral targets (Figure 1A), while test B enabled identification of another seven respiratory viral targets (Figure 1B). The results show discrete band patterns for each of the virus type/subtype-specific amplicons. The QIAxcel DNA High Resolution Kit is capable of resolving amplicons that differ by as few as 5 bp.

It is estimated that, with this assay, 100 samples can be tested within 1 working day. Of that, a maximum of 1 hour is hands-on time. This estimate is based on 30 minutes to prepare the PCR mixture, 3 hours to complete the RT-PCR process, and time for detection using the QIAxcel Advanced System (15 minutes for 12 tests).

The fast and high resolving capacity provided by this experimental setup increases the potential to assess these 16 respiratory virus types/subtypes in future routine surveillance analyses.

Conclusions

- The two-tube assay in combination with the QIAxcel Advanced System and the QIAxcel DNA High Resolution Kit provided a reliable method for respiratory virus identification.
- The assay had sensitivity and efficiency suitable for consideration for routine surveillance.

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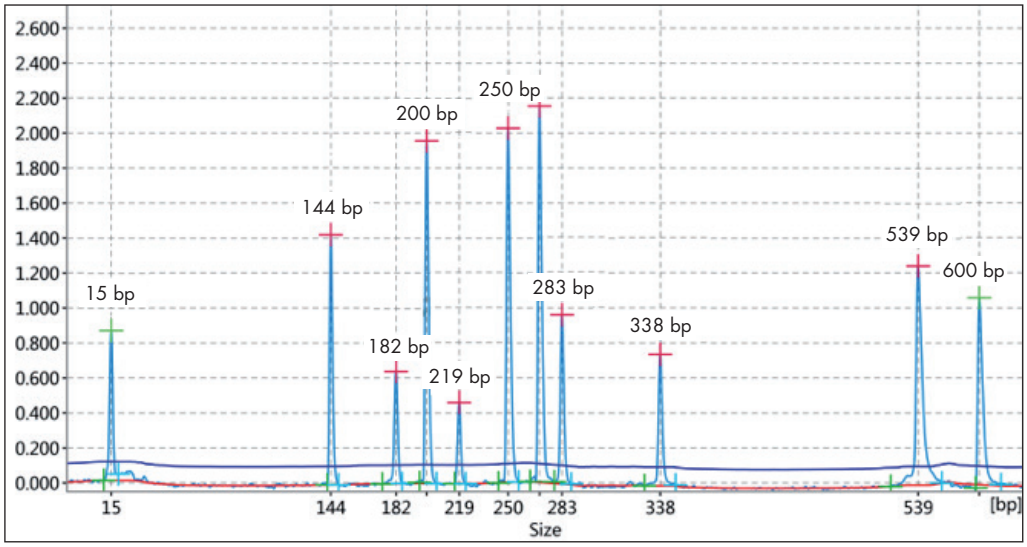

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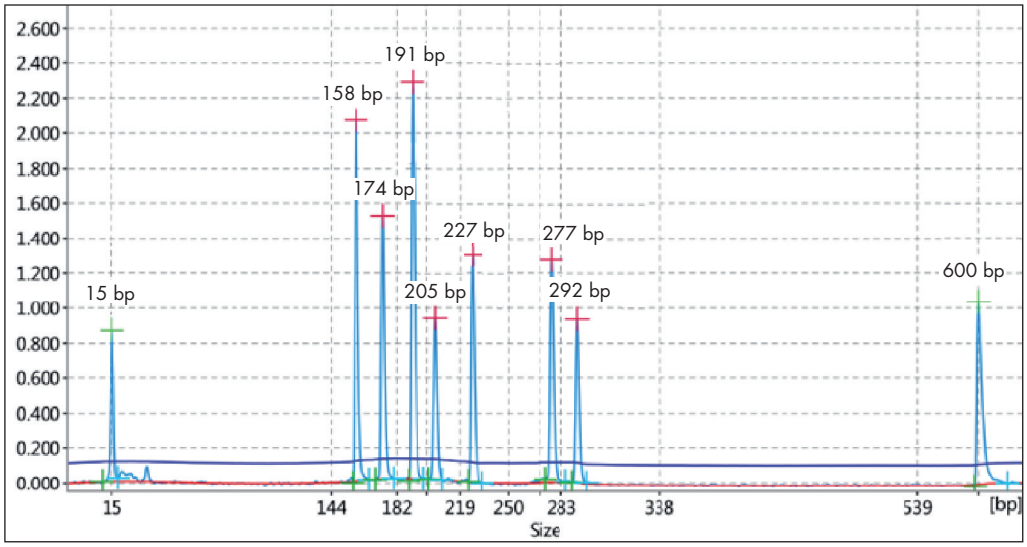

Figure 1. Discrete band identification of 16 respiratory targets with QIAxcel analysis. A Respiratory virus Test A. **B** Respiratory virus Test B. Test peaks are indicated by a red cross; peaks from QX Alignment Marker 15 bp/600 bp are indicated by a green cross.

References

1. Henrickson, K.J. (2004). Advances in the laboratory diagnosis of viral respiratory disease. Pediatr. Infect. Dis. J. **23**, S6.
2. Storch, G.A. (2000) Diagnostic virology. Clin. Infect. Dis. **31**, 739.
3. Li, J., Qi, S., Zhang, C., Hu, X., Shen H., Yang M., et al. (2013) A two-tube multiplex reverse transcription PCR assay for simultaneous detection of sixteen human respiratory virus types/subtypes. Biomed. Res. Int. **2013**, 327620.

Ordering Information

Product	Contents	Cat. no.
QIAxcel Advanced System	Capillary electrophoresis device: includes computer, QIAxcel ScreenGel® Software, installation, training, and 1-year warranty on parts and labor	9002123
QIAxcel DNA High Resolution Kit (1200)	QIAxcel DNA High Resolution Gel Cartridge, Buffers, Mineral Oil, QX Intensity Calibration Marker, 12-Tube Strips	929002
QIAamp Viral RNA Mini Kit (50)	For 50 RNA preps: 50 QIAamp Mini Spin Columns, carrier RNA, Collection Tubes (2 ml), RNase-free buffers	52904
QIAGEN OneStep RT-PCR Kit (25)	For 25 x 50 µl reactions: QIAGEN OneStep RT-PCR Enzyme Mix (1 x 50 µl), 5x QIAGEN OneStep RT-PCR Buffer (1 x 250 µl), dNTP Mix (1 x 50 µl, 10 mM each), 5x Q-Solution (1 x 400 µl), RNase-Free Water (1 x 1.9 ml)	210210
Buffer ATL (4 x 50 ml)	4 x 50 ml lysis buffer for use in purification of nucleic acids using QIAsymphony DSP Virus/Pathogen Kits and the QIAsymphony DSP DNA Mini Kit	939016
QIAsymphony® SP	QIAsymphony sample prep module: includes 1-year warranty on parts and labor	9001297
QIAsymphony DSP DNA Mini Kit (192)	For 192 preps of 200 µl each: Includes 2 reagent cartridges and enzyme racks and accessories.	937236
TopTaq® Master Mix Kit	For 200 x 50 µl reactions: 2x TopTaq Master Mix containing 250 units of TopTaq Polymerase in total, 10x CoralLoad Concentrate, and RNase-Free Water	200403
QX Alignment Marker 15 bp/600 bp (1.5 ml)	Alignment marker with 15 bp and 600 bp fragments	929530
QX DNA Size Marker 25-500 bp (50 µl) v2.0	DNA size marker with fragments of 25, 50, 75, 100, 150, 200, 250, 300, 400, and 500 bp; concentration 100 ng/µl	929560

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