Evaluation of multiple RealStar® (RT-) PCR Kits on various respiratory sample types in a clinical virology laboratory.

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Introduction

Acute respiratory tract infections (common cold to severe respiratory disease) are the most common illnesses in all individuals and constitute more than 60% of respiratory illnesses. A common cold is related to infections of the upper respiratory tract and caused by a viral infection triggered by Rhinovirus (HRV), Coronavirus (CoV) and Respiratory Syncytial Virus (RSV) among other viruses. Cold viruses are transmitted by dispersed droplets from one person to another (aquired by inhalation or physical contacts with infected people).

The common cold differs from the influenza, which is a severe form of the respiratory tract viral infection with additional symptoms like rapidly rising fevers, shivering, body and muscle aches.

Respiratory Syncytial Virus (RSV), Influenza A (Flu A), Influenza B (Flu B), Parainfluenza Virus Type3 (PIV3), Adenovirus (AdV) and the human Metapneumovirus (hMPV) are mainly responsible for serious respiratory diseases.

A rapid, sensitive and effective diagnostisis offers the possibility of an optimized patient management.

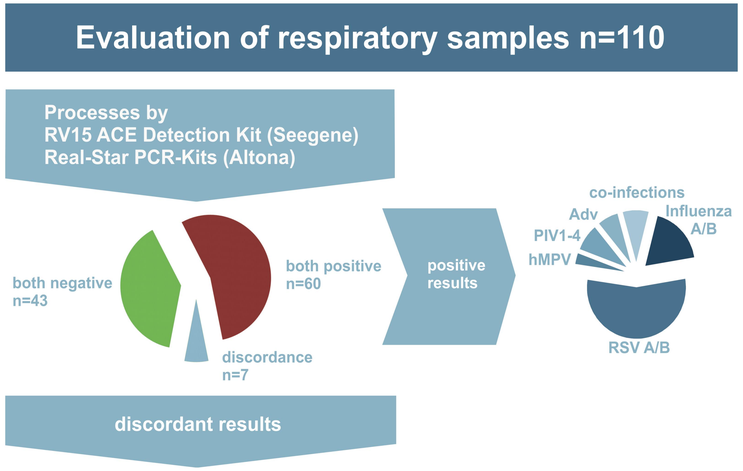
Polymerase-chain-reaction(PCR)-based methods offer increased sensitivity for the detection of most respiratory viruses and should be included into diagnostic testing strategies.

Objectives

The purpose of the study was to evaluate the performance of RealStar (RT-) PCR Kits (altona Diagnostics, Hamburg) for the detection and discrimination of Adenovirus (AdV), Influenzavirus A and B (FLU A/B), Respiratory Syncytial Virus (RSV A and B), Parainfluenzavirus (PIV 1– 4), and human Metapneumovirus (hMPV A and B) after automated nucleic acid extraction from different respiratory sample types using the QIAsymphony SP instrument (QIAGEN Instruments, Hombrechtikon, Switzerland).

The results were compared with the Seegene RV15 ACE Detection Kit routinely used in the laboratory

Results



Parameter	RV15 ACE Detection Kit	Real-Star (RT)-PCR Kit	Quantity	Reference	Results
Influenza A	+	−	1	Paning et al. 2011	✓
Influenza A	−	+	2	Paning et al. 2011	✓
Influenza B	−	+	2	in-house-assay	✓
RSV A	−	+	2	in-house-assay	✓

figure 1: data evaluation

110 respiratory sample matrices were analyzed. 43 were negative with both assays. 55 were positive with both assays (11/55 (20%) Influenza A/B, 33/55 (60%) RSV A/B, 2/55 (3,6%) hMPV A/B, 5/55 (9,1%) PIV1-4, 4/55 (7,3%) AdV). 5 samples showed co-infections.

In total, 103/110 (95,5%) PCR results were concordant on samples extracted by QIAsymphony® SP and detected by both PCR systems.

7 samples showed inconsistent results and were re-analyzed. Positive results could be confirmed by sequencing or other detection systems (figure 1).

Materials and Methods

Various respiratory samples (tracheal secret, pharyngeal swabs, nasopharyngeal secrets and bronchia-alveolar lavages) were processed using the DSP Virus/Pathogen Mini Kit (Qiagen, Hilden) on the automated QIAsymphony® SP instrument (QIAGEN Instruments, Hombrechtikon, Switzerland). The eluates were analyzed in parallel using RealStar (RT-) PCR kits on Rotor-Gene 6000 Instrument (Corbett Research) and Seeplex® RV 15 ACE Detection Kit on the GeneAmp9700 (LifeTechnologies, California) with subsequent analysis using gel electrophoresis system Lab901 TapeStation (Agilent Technologies, California) (figure 2).

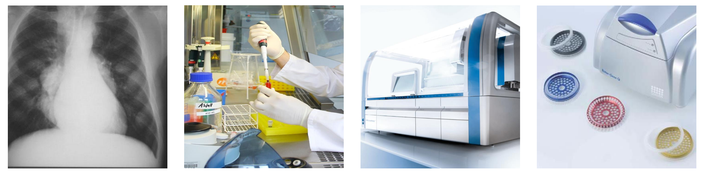


figure 2: respiratory sample preparation and subsequent analysis using real-time PCR

Conclusions

The performance of the RealStar® PCR Kits after sample purification by QIAGEN QIAsymphony® SP was similar and better than the routinely used Seeplex® RV 15 ACE Detection Kit. That means the RealStar (RT-) PCR Kits, in combination with the automated nucleic acid extraction instrument QIAsymphony® SP, are suitable for the analysis of commonly used respiratory sample types in a routine molecular diagnostic laboratory setting.

References

1. Panning M, Baumgarte S, Laue T, Bierbaum S, Raith S, Drexler JF, Helmer A, Falcone-Kapper V, Kochs G, Campe H, Huzly D, Eis-Hübinger AM, Drosten C. Singleplex real-time RT-PCR for detection of influenza A virus and simultaneous differentiation of A/H1N1v and evaluation of the RealStar influenza kit. Journal of Clinical Virology 2011: 171-174.
2. Coiras MT, Aguilar JC, Garcia L, Casas I, Perez-Brena P. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. Journal of Medical Virology 2004: 484-495.