Evaluation of a new Lightmix® multiplex RT-PCR for the detection of 7 respiratory bacterial pathogens

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Abstract

Rapid and accurate diagnosis of bacterial pathogens causing respiratory tract infections is essential for patient care, targeted therapy and application of appropriate infection control measures. Although respiratory pathogens like *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae* and *Legionella* spp. can cause severe pneumonia, diagnostic procedures to detect them are rarely initiated. In addition, due to complexity and poor performance of culture-based and serological detection methods, atypical respiratory pathogens often remain undetected. To enable a fast and accurate detection of the most important atypical bacterial pathogens causing pneumonia, we evaluated the performance of a new respiratory multiplex Lightmix® RT-PCR (TIBMolbiol, Germany). The multiplex RT-PCR displayed a detection limit of 10 DNA copies per RT-PCR reaction and showed 100% sensitivity and 100% specificity, respectively for pathogen detection in 351 clinical respiratory specimens. The Lightmix® multiplex RT-PCR represents a low-cost, fast (< 1.5 hours) and accurate diagnostic tool for respiratory pathogen detection.

Introduction/ Materials and Methods

Community-acquired pneumonia (CAP) is one of the most common infectious diseases. It has been estimated that 15% of all cases of CAP are due to atypical bacterial pathogens[1]. Reliable differentiation between typical and atypical bacterial pathogens causing pneumonia is necessary as they require different therapeutic approaches and antibiotic therapy (e.g. β-lactam resistance in atypical pathogens) [2,3]. Molecular methods, like multiplex RT-PCR, can overcome the limitations of culture-based and serological methods, permitting rapid and sensitive diagnosis, and thereby reducing the fatal consequences of inappropriate antibiotic treatment.

Bacterium	Target
B. parapertussis	*IS1001*
B. pertussis	*IS481*
C. pneumoniae	*ompA*
C. psittaci	*rpoB*
Legionella* spp./ *L. pneumophila	16S rRNA gene
M. pneumoniae	RepMP1

Table 1. Bacterial, respiratory pathogens detected in the multiplex Lightmix® RT-PCR.

Objective

We evaluated a new multiplex Lightmix® RT-PCR for simultaneous detection and identification of 7 important atypical bacterial pathogens (Table 1) in 351 clinical respiratory specimens. Sensitivity and specificity of pathogen detection with the multiplex RT-PCR were compared to singleplex RT-PCR assays (Table 2).

Results

			Singleplex RT-PCR		
B. parapertussis	**Multiplex RT-PCR**		**Negative**	**Positive**	**Specificity = 100%**
		Negative	346	0	**Sensitivity = 100%**
		Positive	0	5	**K = 1**
B. pertussis	**Multiplex RT-PCR**		**Negative**	**Positive**	**Specificity = 100%**
		Negative	183	0	**Sensitivity = 100%**
		Positive	0	168	**K = 1**
C. pneumoniae	**Multiplex RT-PCR**		**Negative**	**Positive**	**Specificity = 100%**
		Negative	348	0	**Sensitivity = 100%**
		Positive	0	3	**K = 1**
C. psittaci	**Multiplex RT-PCR**		**Negative**	**Positive**	**Specificity = 100%**
		Negative	340	0	**Sensitivity = 100%**
		Positive	0	11	**K = 1**
L. pneumophila	**Multiplex RT-PCR**		**Negative**	**Positive**	**Specificity = 100%**
		Negative	331	0	**Sensitivity = 100%**
		Positive	0	20	**K = 1**
***Legionella* spp.**	**Multiplex RT-PCR**		**Negative**	**Positive**	**Specificity = 100%**
		Negative	340	0	**Sensitivity = 100%**
		Positive	0	11	**K = 1**
M. pneumoniae	**Multiplex RT-PCR**		**Negative**	**Positive**	**Specificity = 100%**
		Negative	205	0	**Sensitivity = 100%**
		Positive	0	146	**K = 1**

Table 2. Sensitivity and specificity of the multiplex Lightmix® RT-PCR for detecting atypical respiratory pathogens in clinical specimens and agreement of singleplex RT-PCR vs. multiplex RT-PCR results (ĸ statistics)[4].

References

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CONCLUSION

The multiplex Lightmix® RT-PCR showed very good diagnostic performance for respiratory pathogen detection and is comparable to singleplex RT-PCR assays with respect to specificity and sensitivity.