Journal of Clinical Virology 60 (2014) 141–146

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Evaluation of a multiplex ligation-dependent probe amplification assay for the detection of respiratory pathogens in oncological patients

Lucia Berning[a], Stephan W. Aberle[b], Benedikt Simon[b], Christoph Luger[a], Petra Apfalter[a], Sigrid Machherndl-Spandl[c], Heidrun Kerschner[a,*]

[a] analyse BioLab, Elisabethinen Hospital Linz, Eisenhandstrasse 4-6, 4020 Linz, Austria
[b] Department of Virology, Medical University of Vienna, Kinderspitalgasse 15, 1095 Vienna, Austria
[c] 1st Internal Department – Hematology with Stem Cell Transplantation, Hemostaseology and Medical Oncology, Elisabethinen Hospital Linz, Fadingerstrasse 1, 4020 Linz, Austria

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 18 February 2014
Accepted 26 February 2014

Keywords:
Respiratory tract infection
Oncology
MLPA
Multiplex detection
Time to result
Hands on time

ABSTRACT

Background: Respiratory tract infections are widespread and may cause significant morbidity and mortality in immunosuppressed populations such as oncological patients.
Objectives: The RealAccurate Respiratory RT PCR Kit covering 14 respiratory viruses was compared to the RespiFinder Smart22, a broad-spectrum multiplex ligation-dependent probe amplification (MLPA) test, targeting 22 viral and bacterial respiratory pathogens.
Study design: After verification of its analytical performance, the clinical performance of the RespiFinder Smart22 was evaluated by re-analysis of 96 respiratory samples from oncological patients. Additionally, the time to result (TTR) of both methods was compared.
Results: The analytical performance of the RespiFinder Smart22 fulfilled all previously specified criteria. Concordant results in both assays were achieved in 74.0% of all clinical specimens and in 91.2% when only positive results were taken into account. The RespiFinder Smart22 yielded additional results in a total of 22 (22.9% of 96) samples due to higher test sensitivity and a broader, highly multiplexed spectrum of pathogens. The TTR of a typical routine test consisting of three samples were 206 and 356 min for the RealAccurate Respiratory RT PCR Kit and the RespiFinder Smart22, respectively. However, hands-on time was reduced by 59.0% applying the MLPA method.
Conclusions: In our hands, the RespiFinder Smart22 showed excellent analytical performance while hands-on time was halved in comparison to the RT PCR method. Regarding the clinical evaluation, the MLPA method provided additional results in 22.9% (22/96) of specimens due to its comprehensive format, higher test sensitivity and the capability to detect 22 pathogens compared to 14 with the RealAccurate Respiratory RT PCR Kit.

1. Background

Respiratory tract infections are the most common infections worldwide, with an estimate of two to four infections per year in adults [1]. Especially during the colder season respiratory tract infections occur frequently and may cause significant morbidity and mortality in immunosuppressed populations such as oncological patients [2–4]. The clinical presentation of an infection with respiratory pathogens can range from asymptomatic to severe disease. Considering the broad range of respiratory pathogens it is hardly possible to assign clinical symptoms to a distinct etiology. Hence, molecular diagnostic methods have been established as gold standard and are replacing less sensitive and more

Abbreviations: RT, reverse transcriptase; PCR, polymerase chain reaction; TTR, time to result; InfA/B, influenzavirus A/B; RSVA and B, respiratory syncytical virus A and B; PIV-1, parainfluenzavirus 1; CoV-OC43, coronavirus OC43; Rhv/Entero, rhino-/enterovirus; hMPV, human metapneumovirus; Adv, adenovirus; MLPA, multiplex ligation-dependent probe amplification; Rhv, rhinovirus; HOT, hands on time; QC, quality control; RSD, relative standard deviation; HSCT, hematopoietic stem cell transplantation.

* Corresponding author. Tel.: +43 664 88541589; fax: +43 732 76763686.
E-mail address: heidrun.kerschner@analyse.eu (H. Kerschner).

http://dx.doi.org/10.1016/j.jcv.2014.02.010
1386-6532/

laborious traditional diagnostic methods such as virus isolation by culture or direct immunofluorescence [1,3,5–10]. However, the comprehensive investigation of a respiratory tract infection is time- and resource-consuming when applying singleplex PCR. Therefore, multiplex-based test methods for the detection of numerous respiratory pathogens are entering routine diagnostics [8,9,11,12].

2. Objectives

In the present study a broad spectrum multiplex method, the RespiFinder Smart22 (PathoFinder, Maastricht, The Netherlands), was compared to the routinely used test (RealAccurate Respiratory RT PCR Kit, also PathoFinder) analyzing respiratory samples of oncological patients.

3. Study design

The evaluation of the RespiFinder Smart22 comprised three major parts: the verification of its analytical performance, its routine performance in comparison to the RT PCR method and the evaluation of the time to result (TTR) of both methods. The study was approved by the local ethics committee.

3.1. Molecular assays

The currently used method to detect respiratory pathogens, the RealAccurate Respiratory RT PCR Kit, allows the differentiation of 14 respiratory viruses: influenzavirus A/B (InfA/B), respiratory syncytical virus A and B (RSV A/B), parainfluenzavirus 1 (PIV-1), PIV-2/4, PIV-3, coronavirus OC43 (CoV-OC43) and CoV-229E, rhino-/enterovirus (Rhv/Entero), human metapneumovirus (hMPV) and adenovirus (Adv). The principle of the test is a one-step reverse transcription with subsequent amplification and detection via TaqMan probes on the LightCycler 2.0 platform, requiring a separate master mix for each pathogen. Hence, for analysis of all 14 pathogens in a single clinical specimen 29 capillaries, including all controls, are necessary [13]. The referring physician has the possibility to order individual pathogens as well as the whole panel.

The new method, the RespiFinder Smart22, has been available in Austria since December 2011 and allows the simultaneous differentiation of 22 pathogens. The panel consists of the same viruses as the RealAccurate Respiratory RT PCR Kit, but it covers in addition InfA(H1N1)pdm09, CoV-NL63 and CoV-HKU1, Bocavirus and four bacterial pathogens (*Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila*, and *Bordetella pertussis*). The principle of the test is a multiplex ligation-dependent probe amplification (MLPA) subsequent to a reverse transcription requiring two LightCycler capillaries per specimen.

Prior to the analysis, all samples were extracted with the QIAamp MinElute Virus Spin Kit (Qiagen, Hilden, Germany) according to the instruction for use with a specimen input volume of 200 μl and an output volume of 100 μl for both methods. All samples were stored at −20 °C prior to routine diagnostics with the RealAccurate Respiratory RT PCR Kit to minimize possible effects on the analytical performance and thawed once again for extraction and analysis with the MLPA method.

Clinical samples with discrepant results were retested at the Department of Virology of the Medical University of Vienna with singleplex real time PCRs with detection via TaqMan probes and a nested PCR was employed for the detection of Rhinovirus [14–19].

3.2. Analytical performance

The analytical performance of the RespiFinder Smart22 was verified by testing for accuracy, inter- and intra-assay variability, specificity and sensitivity based on established guidelines [20]. Known positive samples from two different external quality assessment services, QCMD and UKNEQAS that contained Adv, *Bordetella pertussis*, CoV-NL63, hMPV, InfA, InfB, PIV-2, Rhinovirus B Type 72 (Rhv-72), RSV A, and RSV B were tested for the determination of accuracy. The inter- and intra-assay variability were determined by repeatedly analyzing a sample positive for Rhv-72 and a negative sample. To test for specificity, ATCC strains of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were spiked into a previously negative tested pharyngeal wash sample. A dilution series of Rhv-72 and hMPV (undiluted to 1:10,000) was analyzed to compare the sensitivity.

3.3. Clinical performance

In this retrospective study the MLPA method was compared to the RealAccurate Respiratory RT PCR Kit by testing 96 respiratory samples (71.9% pharyngeal washes, 18.8% nasopharyngeal swabs, 9.4% lower respiratory tract samples) of 71 oncological patients treated at the 1st Internal Department of the Elisabethinen Hospital Linz received during the respiratory season of November 2011 to April 2012. After routine diagnostics with the RealAccurate Respiratory RT PCR Kit the original samples were anonymized by assigning a continuous ID number. For analysis with the RespiFinder Smart22 all samples were re-extracted as described in Section 3.1.

3.4. Time to result

The TTR of both methods was evaluated to also elucidate economical aspects on the capability of the MLPA method. It comprises the period of time from the beginning of the analysis of a sample, including the purification of the nucleic acids, to its final result. TTR consists of the hands on time (HOT) and the periods of incubation and was determined for a typical run with three samples by measurement with a stopwatch.

3.5. Data analysis

Data were analyzed with SPSS version 17.0, using descriptive statistics and contingency tables for Fisher's exact test. *P* values of <0.05 were considered significant.

4. Results

4.1. Analytical Performance of the RespiFinder Smart22

For accuracy ten different pathogens were correctly detected in accordance to the results of the external QC. A relative standard deviation (RSD) of <16% was determined for inter-assay variability and an RSD of <6% for intra-assay variability when analyzing Crossing Point-values. For specificity, no cross-reactions were seen when spiking common respiratory bacteria into a negative tested pharyngeal wash sample. Concerning sensitivity, the RespiFinder Smart22 was able to detect Rhv-72 in a 100-fold higher dilution and hMPV with equal sensitivity compared to the RealAccurate Respiratory RT PCR Kit. The results are summarized in Table 4.

4.2. Clinical performance

Demographic and clinical data are summarized in Tables 1 and 2.

Analysis with the RealAccurate Respiratory RT PCR Kit resulted in the detection of a respiratory pathogen in 30 (31.3%) of the 96 samples, whereas retesting with the RespiFinder Smart22 showed positive results in 48 (50.0%) samples ($p = 0.0123$, Fisher's exact test). The comparison of both methods showed matching results in