Evaluation of the new RespiFinder for the detection of respiratory pathogens

P1373

K. Loens[1,4], C. Lammens[1], H. Goossens[1], F. Coenjaerts[2], E. Claas[3], A. van Loon[2], M. Ieven[1,4] for the GRACE study team
[1] Department of Medical Microbiology, VAXINFECTIO, University of Antwerp, Antwerp, Belgium, [2]UMC-UTRECHT, Utrecht, The Netherlands, [3]LUMC, Leiden, The Netherlands, [4]Belgian Reference Centre for Respiratory Pathogens, University Hospital Antwerp, Antwerp, Belgium

Laboratory of Medical Microbiology
Vaccine & Infectious Disease Institute
University of Antwerp

Genomics to combat Resistance against Antibiotics in Community-acquired LRTI in Europe

TRACE
Translational Research on Antimicrobial resistance and Community-acquired infections in Europe

OBJECTIVES

We investigated the aetiology in lower respiratory tract infections in the European GRACE primary care network (PCN) using mono- and small multiplex real-time nucleic acid amplification tests. These had been shown to be more sensitive than some multiplex (MX) assays on a GRACE proficiency panel, but are more time-consuming and expensive due to the large diversity of respiratory pathogens. Large MX assays could be more convenient. This study compares the performance of a new RespiFinder (PathoFinder) kit to in-house real-time PCRs on a selection of positive and negative respiratory specimens.

RESULTS

210 and 216 (incl. 5 HKU1) respiratory pathogens were detected by the real-time in-house PCRs and the RespiFinder, respectively. Sensitivity and specificity of the commercial assay is shown in the table: atypical bacteria were detected significantly less frequently by the RespiFinder compared to in-house PCRs. INF A, RSV and HMPV were detected more often by the RespiFinder compared to in-house PCRs. All other sensitivities were not significantly different. In general, samples found negative by the commercial assay tended to have a low viral load (based on Ct-value).

Correspondence:
Katherine Loens
Katherine.loens@uza.be
University Hospital Antwerp, Wilrijkstraat 10, B-2650 Edegem, Belgium

MATERIALS & METHODS

190 nasopharyngeal flocked swabs and 5 sputa (*L. pneumophila*) were selected from a biobank containing respiratory specimens collected prospectively in 12 PCNs in 8 European countries during 3 winter seasons. They were sent to the central lab for subsequent nucleic acid (NA) extraction by the NucliSens EasyMag. Aliquots of NA extracts were sent to LUMC and UMCUTRECHT for detection of influenzaviruses (INF) A/B, parainfluenzavirus (PIV) 1-4, human rhinoviruses (HRV), human metapneumovirus (hMPV), respiratory syncytial virus (RSV), adenoviruses (HAdV), and coronaviruses (HCoV 229E, OC43 and NL63) by in-house monoplex and small MX real-time PCRs. In Antwerp, PCR for detection of *M. pneumoniae*, *C. pneumoniae*, *B. pertussis* and *L. pneumophila* was applied. The RespiFinder was retrospectively blind applied by PathoFinder after local nucleic acid extraction without sample pretreatment. Sensitivity and specificity were calculated against in-house PCRs.

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CONCLUSIONS

For most pathogens, in-house PCRs are equally sensitive when compared to the RespiFinder. This new version of the RespiFinder might be an alternative to reduce hands-on time and detects in addition HCoV HKU1. The low sensitivity for *L. pneumophila* may be caused by the high viscosity of the untreated sputa.

Table 1. Overview of organisms detected by in-house real-time PCRs and the new RespiFinder

Organism	Nr of organisms detected by In-house Real-Time PCR	Nr of organisms detected by RespiFinder®	RespiFinder Sensitivity	RespiFinder Specificity
M. pneumoniae	6	5	66.7	99.5
C. pneumoniae	12	9	75.0	100
B. pertussis	16	11	62.5	99.4
L. pneumophila	5	1	20	100
HAdV	5	4	80	100
INFA	30	35	100	97.0
INF H1N1	15	17	100	98.9
INFB	15	15	100	100
HCoV	14	16	92.9	98.3
HKU1	ND	5		
hMPV	16	21	93.8	96.7
HRV/ENT	17	19	94.1	98.3
RSVA	15	19	93.3	97.2
RSVB	16	18	93.8	98.3
PIV1-4	20	16	75.0	99.4
HBoV	5	5 (type 1)	60.0	99.0
TOTAL	210	211	86.5	98.8

Table 2. Ct-values RespiFinder-/PCR+ samples

Organism	Ct in-house PCR
M. pneumoniae	37,62
C. pneumoniae	36,80; 35,92; 27,03
B. pertussis	36,51; 36,34; 37,19; 37,05; 37,04; 36,51
HAdV	38,00
HBoV	37,00; 38,00

Organism	Ct in-house PCR
HRV	38,00
PIV 1-4	39,00; 43,00; 37,00; 37,00
RSV A/B	33,00; 34,00
HMPV	31,00
HCoV	34,00