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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.

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.

Results: 207 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).

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.

| Organism | Nr of organisms detected by | | RespiFinder | |
|-----------------------|-----------------------------|--------------|-------------|-------------|
| | In-house Real-Time PCR | RespiFinder® | Sensitivity | Specificity |
| <i>M. pneumoniae</i> | 6 | 5 | 66.7 | 99.5 |
| <i>C. pneumoniae</i> | 12 | 9 | 75.0 | 100 |
| <i>B. pertussis</i> | 16 | 11 | 62.5 | 99.4 |
| <i>L. pneumophila</i> | 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 | 207 | 216 | 86.5 | 98.8 |