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# Clinical implications of rapid eplex® respiratory pathogen panel testing compared to laboratory developed real-time pcr

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## ABSTRACT

**Introduction:** Rapid diagnosis of respiratory infections is of great importance for adequate isolation and treatment. Due to the batch-wise testing, lab developed real-time PCR assays (LDT) often result in a time to result of one day. Here, LDT was compared with rapid ePlex® respiratory pathogen (RP) panel testing of GenMark Diagnostics (Carlsbad, CA, USA) with regard to time to result, installed isolation precautions and antibacterial/antiviral treatment.

**Methods:** Between January and March 2017, 68 specimens of 64 patients suspected of an acute respiratory infection were tested with LDT and ePlex® RP panel. Time to result was calculated as time between sample reception and result reporting. Information regarding isolation and antibacterial/antiviral treatment was obtained from the patient records.

**Results:** Thirty specimens tested LDT positive (47%) and 29 ePlex® RP panel positive (45%). The median time to result was 27.1 hours (range 6.5-96.6) for LDT vs. 3.4 hours (range 1.5-23.6) for RP panel, *P*-value <0,001. In 14 out of 30 patients, isolation was discontinued based on ePlex® RP panel results, saving 21 isolation days. ePlex® RP panel test results were available approximately one day ahead of LDT results in the 19 patients receiving antiviral/ antibacterial treatment. In addition, two bacterial pathogens, not requested by the physician, were detected using RP panel.

**Conclusions:** Analysis of respiratory infections with the ePlex® RP panel resulted in a significant decrease in time to result, enabling a reduction in isolation days in half of the patients. Furthermore, syndromic RP panel testing increased identification of causative pathogens.

## INTRODUCTION

Respiratory tract infections are a leading cause of hospital admission, morbidity, and mortality[1-4]. At presentation aetiological agents of the respiratory tract infection cannot be identified solely based on clinical signs and symptoms. Therefore, and awaiting microbiological confirmation, empirical antibiotic and antiviral treatment is initiated based on severity score and the influenza season[5]. Since only a minority of the infections is being caused by bacteria, this empiric antibiotic treatment approach is redundant and can lead to an increase in antibiotic resistance. Moreover, empiric isolation precautions are installed to protect other patients and health care workers from a possible (viral) infection. Altogether, there is a need for rapid identification or exclusion of a viral respiratory tract infection to reduce inappropriate (unnecessary) hospital hygienic interventions and focus (shorten) antibacterial/antiviral treatment.

Currently, the diagnosis of respiratory infections is usually based on (a combination of) molecular amplification methods and bacterial culture. In our laboratory, lab developed real-time PCR multiplex assays (LDTs) are used that show excellent sensitivity and specificity. However, this approach is limited by the number of targets per multiplex reaction and the need for batch-wise testing. The assays are performed once daily, with a time to result of approximately 20 hours.

Recently, the Respiratory Pathogen (RP) Panel of GenMark Diagnostics (Carlsbad, CA, USA) has become available for detection of an extensive panel of respiratory pathogens (21 respiratory viruses, 3 bacterial species, see Methods) using eSensor technology[6]. This test is a cartridge based molecular assay to be used on the ePlex® platform with a time to result of approximately 90 minutes that showed an concordance of >97% compared to LDT[7]. Hypothetically ePlex® RP panel testing represents a considerable reduction in time to diagnosis, as compared to LDT, which could have significant clinical benefits. In this paper, a pilot study is reported that analysed the implications of using the ePlex® RP panel for the detection of respiratory infections compared to LDT regarding time to result, isolation precautions, and antibacterial/antiviral therapy.

## METHODS

### Inclusion of patients

This prospective, single centre study in the Leiden University Medical Center (LUMC) included patients from January to March 2017. Patients with symptoms of an acute respiratory infection were included upon request of the physician of the acute ward, intensive care unit, and paediatric department. Specimens included were obtained during weekdays and tested with both the RP panel and the LDTs after consulting the microbiologist. Information regarding baseline characteristics, infection parameters, admittance, isolation and treatment was obtained from the electronic patient records. Additional information about cultures was retrieved from the laboratory information system (GLIMS, MIPS, Belgium). The medical ethics review committee of the LUMC approved the study.

**Primary outcome measure**

The primary endpoint of this study was the time to result of the ePlex® RP panel compared to the LDT.

**Secondary outcome measures**

The ePlex® RP panel was offered as a pilot to elevate the pressure on droplet isolation rooms, thus isolation was discontinued based on the ePlex® RP panel results. Due to the pilot nature of this study antibacterial and antiviral treatment were not adjusted based on the ePlex® RP panel results, therefore only the theoretical time reduction in treatment was calculated using the time to results of the ePlex® RP panel and the LDT. Secondary outcome measures were the reduction of isolation days based on ePlex® RP panel ahead of LDT results, the theoretical reduction in hours in oseltamivir and atypical pneumonias treatment calculated with the time to results of LDT and ePlex® RP panel, and possible additional diagnosis found with the ePlex® RP panel.

**Laboratory-developed test (LDT)**

LDT viral testing and testing for *Mycoplasma pneumoniae, Chlamydia pneumoniae and Chlamydia psittaci* was performed the same day on all samples that arrived at the laboratory before 8:15 A.M. Samples arriving at the laboratory before 3:30 P.M., were tested for *Legionella pneumophila* and *Bordetella parapertussis* the following day. These assays were performed daily from Monday till Friday and on request on weekend days. The viral respiratory panel of LDT consists of adenovirus, bocavirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, coronavirus OC43, influenza A, influenza B, human metapneumovirus, parainfluenza 1-4 (differentiation with differently labelled probes), respiratory syncytial virus, and rhinovirus. In addition, testing for bacterial pathogens could be requested: Legionella species, *Legionella pneumophila*, *Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia psittaci, Bordetella pertussis,* and *Bordetella parapertussis.*

All sputa samples were 1:5 diluted in PBS and homogenized by bead-beating prior to extraction. Then, 200 µl of each respiratory sample was used to extract 100 µl total nucleic acids using the Total Nucleic Acid extraction kit on the MagnaPure LC system (Roche Diagnostics). Nucleic acid amplification and detection by real-time PCR was performed on a BioRad CFX96 thermocycler, using primers, probes and conditions as described previously[8-10]. For the detection of *Mycoplasma pneumoniae, Chlamydia pneumoniae,* and *Chlamydia psittaci* the b-CAP assay (Biolegio, Nijmegen, the Netherlands) developed for the BD-max system was used by testing 200 µl of each respiratory sample according to the manufacturer's instructions[11]. LDT test results were reported in the electronic patient record. Time to result for the LDT was calculated as time of receipt of the sample in the laboratory to the time results were available in the electronic patient record.

**ePlex® RP panel**

Specimens for diagnosis using the CE-IVD cleared RP panel were accepted on weekdays between 8:15 A.M. and 3:00 P.M. and tested during the day, as soon as possible. The ePlex® respiratory panel was not offered during the weekend, while treatment was not adjusted based on the results. The RP panel as used in the study was able to detect: adenovirus, bocavirus, coronavirus 229E, coronavirus HKU1, coronavirus NL63, Coronavirus OC43, influenza A H1, influenza A 2009 H1N1, influenza A H3, influenza B, metapneumovirus, Middle East respiratory syndrome coronavirus, parainfluenza 1-4, respiratory syncytial virus A and B, rhinovirus/ enterovirus, *Bordetella pertussis, Legionella pneumophila,* and *Mycoplasma pneumoniae.* As with LDT, sputa samples were diluted in an 1:5 dilution using PBS. According to the manufacturer's instructions 200 μl of the respiratory sample was pipetted in a buffer tube and after vortexing transferred to the ePlex cartridge and subsequently to the ePlex tower. If the test gave an invalid result, the run was repeated. Results were reported by telephone to the requesting physician, since the results were not reported in the electronic patient record. Time to result was calculated as time of receipt of the sample to the time results were reported by telephone.

**Statistics used for comparison**

Time to result was compared with Wilcoxon Signed Rank Test, using IBM SPSS Statistics version 23 software for Windows. A P-value <0.05 was considered statistically significant.

**RESULTS**

**LDT and ePlex® RP panel results**

Between January and March 2017 64 patients were included with symptoms of acute respiratory infection whose characteristics are summarized in Table 1. A total of 68 samples were tested, comprising 40 throat swabs, 13 sputum samples, 11 nasal lavages, and four nasopharyngeal swabs. Thirty-four tested positive for a respiratory pathogen in one or both assays. Six samples failed in the ePlex® RP panel, of which two gave a valid result upon retesting. The other four were not retested, two because of insufficient remaining sample volume. The failed samples, if not retested, were excluded from further analysis, leaving 64 samples of 61 patients for further analysis. None of the samples failed in the LDT.

Of the 64 samples 31 tested positive for a total of 37 pathogens with LDT or ePlex® RP panel (Table 2). Using LDT, 30 tested positive and 34 negative, whereas this was 29 and 35 using the ePlex® RP panel. As shown in Table 3A, a discordant result was found in five samples.

In three patients, different sample types were tested (Table 3B). From the first a sputum and a throat swab were collected, of which only the first tested LDT positive for influenza A. The second tested rhinovirus positive in a nasal lavage, with LDT only, and negative in sputum. Of the third patient a sputum and a throat swab were tested, of which only the sputum tested coronavirus 229E positive.

**Table 1. patient characteristics**

| | Patients n=64 | Range/% |
|---|---|---|
| **Demographics** | | |
| **Age, median years (range)** | 60 | 0-93 |
| **Male sex (%)** | 33 | 52 |
| **Clinical features** | | |
| **Diagnosis** | | |
| **Pneumonia (%)** | 25 | 39 |
| **COPD/ asthma exacerbation (%)** | 7 | 11 |
| **RTI other than pneumonia (%)** | 12 | 19 |
| **Other diagnosis (%)** | 20 | 31 |
| **Leukocytes, median x10$^9$/L (range)** | 11.4 | 0.44-49.16 |
| **C-reactive protein level, median mg/L (range)** | 62 | 2-360 |
| **Cough (%)** | 49 | 77 |
| **Sputum (%)** | 26 | 41 |
| **Previous antibiotic treatment (%)** | 20 | 31 |
| **Duration of symptoms, median days (range)** | 2 | 1-21 |
| **Comorbidity** | | |
| **COPD/asthma (%)** | 17 | 27 |
| **Diabetes (%)** | 7 | 11 |
| **Malignancy (%)** | 6 | 9 |
| **Transplantation (%)** | 12 | 19 |
| **Auto-immune disease (%)** | 8 | 13 |
| **Admission ward** | | |
| **Acute ward** | 32 | 50 |
| **Intensive care (including children)** | 8 | 13 |
| **Paediatric department** | 8 | 13 |
| **Other departments** | 15 | 23 |
| **Not admitted** | 1 | 1 |

Abbreviations: n, number; COPD, chronic obstructive pulmonary disease; RTI, respiratory tract infection

**Table 2. Respiratory pathogens found in clinical samples with lab developed real-time PCR assay or ePlex® RP panel**

| Pathogens | LDT | ePlex® RP panel |
|---|---|---|
| Coronavirus 229E | 2 | 2 |
| Coronavirus HKU1 | 1 | 1 |
| Human bocavirus | 1 | 1 |
| Human metapneumovirus | 5 | 4 |
| Influenza A | 10 | 9 (all H3) |
| Influenza B | 1 | 1 |
| Parainfluenza virus type 3 | 1 | 1 |
| Respiratory syncytial virus | 4 | 0 |
| Respiratory syncytial virus type A | | 2 |
| Respiratory syncytial virus type B | | 2 |
| Rhinovirus/enterovirus | 8 | 9 |
| *Bordetella pertussis* | 1 | 1 |
| *Mycoplasma pneumoniae* | 1 | 1 |

Abbreviation: LDT, Lab developed real-time PCR

**Table 3A. Discrepant results of lab developed real-time PCR assay compared to ePlex® RP panel**

| | LDT | ePlex® RP panel |
|---|---|---|
| Throat swab | Negative (retesting negative) | RV/EV |
| Nasopharyngeal swab | InfA (Cq 26) (enterovirus negative) | InfA- RV/EV |
| Nasal lavage | RV (Cq 39.1) | Negative |
| Sputum | MPV (Cq 30.3) | Negative (retesting MPV pos) |
| Sputum | infA (Cq 33.1) | Negative (retesting negative) |

Abbreviations: LDT, Lab developed real-time PCR; RV, rhinovirus; EV, enterovirus; InfA, influenza A; MPV, metapneumovirus; Cq,quantification cycle

**Table 3B. Different sample types tested**

| Patient | Material | LDT | ePlex® RP panel |
|---|---|---|---|
| 1 | Sputum | infA (Cq 33.1) | Negative |
| | Throat swab | Negative | Negative |
| 2 | Sputum | Negative | Negative |
| | nasal lavage | RV (Cq 39.1) | Negative |
| 3 | Sputum | CoV 229E (Cq 33.4) | CoV229E |
| | Throat swab | Negative | Negative |

Abbreviations: LDT, Lab developed real-time PCR; RV, rhinovirus; InfA, influenza A; CoV,coronavirus; Cq,quantification cycle

**Chapter 4**

**Primary outcome measure:**

**Difference in time to result**

For 62 of the 64 samples, both the time of acceptance and the time of result was recorded. The calculated time to result was significantly shorter, approximately 24 hours, for the ePlex® RP panel than for LDT (*P*<0.001) (Table 4). A time to result of over 35 hours was seen with LDT testing in 15 samples, of which 13 had arrived on Friday and were tested on Monday. In the two remaining samples there was a delay in requesting and authorisation of the test subsequently. In the ePlex® RP panel, four samples had a time to result of more than 18 hours. Two of these samples were already at the laboratory for several hours before the ePlex® RP panel testing was requested, while the testing of two sample was requested after 3:00 P.M. and therefore performed the next day (one due to failure of the initial sample).

**Table 4. Time to result in hours of lab developed real-time PCR assay compared to ePlex® RP panel**

| Time to result | LDT | ePlex® RP panel | P-value[*] |
|---|---|---|---|
| Median (hours) | 27.11 | 3.35 | <0,001 |
| Range (hours) | 6.52-96.57 | 1.45-23.56 | |

Abbreviation: LDT, Lab developed real-time PCR
[*] P-value calculated with Wilcoxon signed rank test

**Secondary outcome measures**

**Consequences for patient isolation**

Of the 61 patients included in the analysis, 60 were admitted to the hospital at the time respiratory testing was requested. Fifty-one of these hospitalised patients were isolated while awaiting test results, whereas nine patients were not admitted in isolation. In these cases isolation was not installed mainly because of low clinical suspicion of a pathogen requiring isolation. One of these nine patients needed isolation, since the ePlex® RP panel tested positive for influenza A (three days ahead of LDT).

The tests showed that 19 out of 51 patients admitted in isolation had a respiratory pathogen requiring isolation. Of the remaining 32 patients, one died before test results became available and for one patient the duration of isolation was unknown, leaving 30 patients for further analysis. In 14 of these isolation was discontinued based on ePlex® RP panel results ahead of LDT results. This resulted in a total reduction of 21 isolation days, with a median reduction of 2 days (range 1-4 days) per patient. In eight of the remaining patients, isolation was discontinued when LDT results became available. In the other eight patients, of which three children, isolation was not withdrawn at the moment LDT results were reported.

**Theoretical consequences for antiviral and antibacterial treatment**

A total of 50 out of the 61 patients received antiviral or antibacterial treatment during hospitalization. Oseltamivir treatment was initiated in 19 patients awaiting test results, of which five tested positive for influenza A. In the 14 influenza ePlex® RP panel negative patients, oseltamivir could have been stopped approximately one day earlier (median of 22.59 hours, range 5.33-72.03) based on ePlex® RP results compared to LDT (Table 5). Of the in total 11 patients who tested influenza positive, the remaining six did not receive oseltamivir at the time of diagnosis. In one patient, oseltamivir treatment was started as soon as ePlex® RP panel showed influenza A, one day prior to LDT results, and one patient started when LDT was positive. Four patients did not receive any antiviral treatment, of which two were already dismissed at the time of definite LDT diagnosis.

Awaiting test results, 19 patients received antibiotic treatment for bacteria causing atypical pneumonias. In none of these patients, either the ePlex® RP panel or the LDT (eight were tested) was positive for *Bordetella pertussis, Legionella pneumophila,* and *Mycoplasma pneumoniae.* In theory, in these 19 patients, a median duration of 23.35 hours (range -0.43- 75.28 hours) antibiotic treatment for atypical pneumonia could have been saved, if treatment was stopped when ePlex® RP panel tested negative.

**Chapter 4**

**Table 5. Theoretical median time in hours of isolation and treatment calculated based on time to results**

| | No. | LDT (range) | ePlex® RP panel (range) | Difference (range) |
|---|---|---|---|---|
| **Oseltamivir, h** | 14 | 27.08 (10.10-75.15) | 3.38 (2.00-23.56) | 22.59 (5.33-72.03) |
| **Antibiotics atypical pneumonias, h** | 19 | 27.12 (8.27-81.11) | 3.38 (1.52-23.56) | 23.35 (-0.43-75.28) |

Abbreviations: No, number of patients; LDT, Lab developed real-time PCR; h, hours

**Additional diagnoses**

Of the 61 patients, two tested positive by ePlex® RP panel for a bacterial agent, one *Bordetella pertussis* and one *Mycoplasma pneumoniae.* In both patients, testing for these pathogens was not requested for by the clinician and as a consequence not included in the routine diagnostic LDT workflow. The positive ePlex® RP panel results were confirmed by LDT with Cq-values of 25.6 and 34.6 for *B. pertussis* and *M. pneumoniae*, respectively. LDT testing for atypical bacterial pathogens (*Mycoplasma pneumoniae, Chlamydia pneumoniae, Chlamydia psittaci)* was requested in only 16 patients. Legionella LDT testing was requested in only 10 patients.

## DISCUSSION

As hypothesized, diagnosis with the ePlex® RP assay significantly reduced the time to result (median 23.34 hours) as compared to batch wise LDT testing. Consequently, a total of 21 isolation days were saved and three days of influenza A exposure prevented. Unnecessary oseltamivir treatment could have been shortened at least 20 hours in 14 patients and antibiotic treatment for atypical pneumonias by a median of 23.35 hours days in 19 patients. Proper therapeutic and isolation measurements could be installed in two patients for bacterial pathogens based on ePlex® RP panel detection that were not considered by the treating physicians and therefore not analysed by routine LDT.

To our knowledge this study is the first to report the use of the ePlex® respiratory panel in a clinical setting. It demonstrated a significant time reduction, reflecting previous clinical studies implementing rapid molecular testing[12-15], and significantly reduced the number of isolation days. Furthermore, confirmation of a single viral cause of infection in a cohort of patients enabled cohort nursing, which increased the number of isolation rooms available to patients awaiting identification of their respiratory pathogen. Efficient use of isolation rooms is essential during influenza season when the demand for these rooms is high.

The rapid ePlex® RP panel results could have resulted in a reduction of oseltamivir usage, which is in line with previous studies[14]. Results regarding reduction in antibiotic treatment for atypical pneumonias should be interpreted with care, while they are, according to the Dutch guidelines, only indicated for *Legionella pneumophila* in high risk populations and can also been stopped based on negative urine-antigen testing. The lack of routine testing for atypical respiratory bacterial pathogens (mostly *Legionella pneumophila*) and the finding of additional respiratory pathogens, initially not considered by the clinicians, underline the importance of syndromic respiratory testing.

Our study has a number of limitations. First, the clinical impact of our pilot study was hampered by its design. Since the ePlex® RP assay was readily offered to reduce the quest for isolation rooms during the coinciding influenza and RSV epidemics early 2017, its test results were not shown yet in the hospital information system but reported by phone, creating a bias. Moreover, the ePlex® RP panel result was reported as a provisional result awaiting routine LDT confirmation. The delay in showing the test results in the electronic patient record, might have withheld clinicians to discontinue isolation and therefore created an underestimation of the true clinical potential. Furthermore, the findings of this study cannot be extrapolated readily, since this was a single centre study during just a part of one winter season. The benefits of rapid diagnostics might be more pronounced when assessing complete respiratory seasons.

So far, the ePlex® RP panel has been CE-IVD cleared for nasopharyngeal swabs only. However, especially samples from the lower respiratory tract as sputum and bronchoalveolar lavage can be important to include in the CE-IVD clearance, since our study shows that these samples might have a higher diagnostic yield. However, both in our previous and current study several different sample types were tested with good results[7]. Nevertheless, the ePlex® RP panel had a failure rate of nearly 10%, in two cases due to internal control failure, none of the LDT tests failed. Overall, the ePlex® RP

panel results showed excellent concordance with our LDT, only three LDT positives (all with Cq-values >30) could not be detected using the ePlex® RP panel. This is in line with our previous findings reported by Nijhuis et al.[7]. The ePlex® RP panel is based on syndromic testing and has a standard panel containing most common respiratory pathogens that are requested by the physician. However, the ePlex® RP panel is not complete, especially when caring for in immunocompromised patients. In that case additional LDT testing for Legionella species, cytomegalovirus, herpesvirus, toxoplasmosis and fungal pathogens would still be necessary. Compared to LDT, ePlex® RP panel testing is more expensive regarding reagents and consumables but cheaper with respect to hand-on-time.In addition, rapid diagnostics will result in a cost reduction in the clinical departments as demonstrated previously[16].

In conclusion, diagnosis of respiratory infections with the ePlex® RP assay resulted in a significant reduction in time to result compared to LDT, which causes a reduction in isolation days and theoretically improved treatment regimens. Because of these advantages, we assume this rapid diagnostic molecular assay will be of added value for ongoing improvement in patient care.

**Chapter 4**

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