Comparative Evaluation of Six Commercialized Multiplex PCR Kits for the Diagnosis of Respiratory Infections

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Abstract

The molecular diagnosis of respiratory infection can be performed using different commercial multiplex-based PCR kits whose performances have been previously compared individually to those of conventional techniques. This study compared the practicability and the diagnostic performances of six CE-marked kits available in 2011 on the French market, including 2 detecting viruses and atypical bacteria (from Pathofinder and Seegene companies) and 4 detecting only viruses (from Abbott, Genomica, Qiagen and Seegene companies). The respective sensitivity, specificity, accuracy and agreement of each multiplex technique were calculated by comparison to commercial duplex PCR tests (Argene/bioMérieux) used as gold standard. Eighty-eight respiratory specimens with no pathogen (n = 11), single infections (n = 33) or co-infections (n = 44) were selected to cover 9 viruses or groups of viruses and 3 atypical bacteria. All samples were extracted using the NUCLEISENS easyMAG® instrument (bioMérieux). The overall sensitivity ranged from 56.25% to 91.67% for viruses and was below 50% with both tests for bacteria. The overall specificity was excellent (>94% for all pathogens). For each tested kit, the overall agreement with the reference test was strong for viruses (kappa test >0.60) and moderate for bacteria. After the extraction step, the hands-on time varied from 50 min to 2h30 and the complete results were available in 2h30 to 9 h. The spectrum of tested agents and the technology used to reveal the PCR products as well as the laboratory organization are determinant for the selection of a kit.

Introduction

The global burden of acute respiratory infection (ARI) remains a huge problem of Public Health. In developed countries, the number of viral respiratory episodes per year has been estimated between 6 and 10 in children before school age versus 3 to 5 in those after this age and ARI represents the cause of 30 to 40% of hospitalization [8], (iii) the rapid implementation of isolation measures when necessary, thus limiting the risk of nosocomial transmission, (iv) the collection in real time of new epidemiological data on the seasonal spread of pathogens, and (v) the identification of simultaneous or successive infections [6], [9–10] that may justify specific intervention or explain the severity of the clinical picture. The detection of agent’s genome responsible for respiratory infection have been revolutionized by recent advances in the field of nucleic acid amplification tests and notably of multiplex PCR [4], [11–14]. In addition to their excellent sensitivity, much superior to that of conventional techniques [15], these techniques allow the simultaneous detection of a wide range of pathogens, mostly viruses [16–19], but also atypical bacteria (including Mycoplasma pneumoniae, Chlamydia pneumoniae, L. pneumophila and Bordetella pertussis) [20–23], with a short-time return of the results to clinicians.

The need for precise and rapid identification of the causative agents of ARI has been reviewed recently [2], [5–6]. The main advantages of this strategy are (i) a better use of antimicrobials including antiviral drugs and antibiotics [3], [7] and thus limiting the development of bacterial resistance, (ii) the reduction of unnecessary paraclinical explorations and of the duration of hospitalization [8], (iii) the rapid implementation of isolation measures when necessary, thus limiting the risk of nosocomial transmission, (iv) the collection in real time of new epidemiological data on the seasonal spread of pathogens, and (v) the identification of simultaneous or successive infections [6], [9–10] that may justify specific intervention or explain the severity of the clinical picture. The detection of agent’s genome responsible for respiratory infection have been revolutionized by recent advances in the field of nucleic acid amplification tests and notably of multiplex PCR [4], [11–14]. In addition to their excellent sensitivity, much superior to that of conventional techniques [15], these techniques allow the simultaneous detection of a wide range of pathogens, mostly viruses [16–19], but also atypical bacteria (including Mycoplasma pneumoniae, Chlamydia pneumoniae, L. pneumophila and Bordetella pertussis) [20–23], with a short-time return of the results to clinicians.

The objective of the present study was to evaluate the technical performances of six commercial kits based on multiplex PCR and available on the European market at the beginning of 2011 for the diagnosis of respiratory infection. The kits were compared to a
combination of biplex PCR tests used as gold standard on a panel of respiratory secretions selected for their content in various infectious agent(s). The performances of each kit for detecting different respiratory pathogens, including sensitivity, specificity, accuracy and agreement, were evaluated for each agent; other technical properties were also taken into consideration. Globally, the kits whose commercial development is still pursued were shown to be convenient for routine use in a clinical laboratory setting.

**Materials and Methods**

**Presentation of the Kits used in this Study**

Six kits based on multiplex detection of respiratory viruses or atypical bacteria and available in the French market in 2011 were tested. All were used as recommended by the manufacturers. The technologies are depicted in Figure 1. Letters A to F were used to design the kits in the following sections. Kit A corresponds to RespiFinder® SMART 22 (Pathofinder, Maastricht, The Netherlands); the reverse transcription and preamplification steps were performed on GeneAmp® PCR system 2700 (Applied Biosystems) and the hybridization, ligation and detection steps on the LightCycler® 480 system (Roche Applied Science). Kit B corresponds to the Seeplex® RV15 OneStep ACE Detection and Pneumobacter ACE Detection (Seegene Inc., Seoul, South Korea); a GeneAmp® PCR system 9700 (Applied Biosystems) was used for amplification and the size of PCR products was read on a TapeStation after electrophoresis using ScreenTape. Kit C corresponds to the Magicplex RV Panel Real-time Test (Seegene Inc); the amplification was performed using GeneAmp® PCR system 9700 (Applied Biosystems) and the detection of PCR products was done on ABI7500 (Applied Biosystems). Kit D corresponds to Clart® Pneumovir (Genomica, Madrid, Spain); the amplification was performed on GeneAmp® PCR system 2700 (Applied Biosystems) and the hybridization of PCR products on the array was read using the Clinical Array System (CAR) (Genomica). Kit E corresponds to xTAG® respiratory Viral Panel fast (Abbott, Rungis, France) and kit F to ResPlex II Panel v2.0 (Qiagen, Hilden, Germany), for these two kits, the amplification was performed on GeneAmp® PCR system 9700 (Applied Biosystems) and the detection of PCR products on a Luminex platform (Bio-Plex, Bio-Rad).

The test used as gold standard for evaluating the six kits described above corresponds to a combination of 7 duplex Respiratory Multi Well System r-geneTM (Influenza A/B, RSV/hMPV, Rhino&EV/CC, AD/hBoV, Chla/Myco pneumo, HCoV/HPIV and Bordetella) commercialized by Argene/bioMérieux (Marcy l’Etoile, France). The real-time PCR reactions were performed on an ABI7500fast (Applied Biosystems).

The viruses and/or bacteria that could be detected by the 7 kits mentioned above are listed in Table 1.

**Selection and Preparation of the Respiratory Samples**

In order to constitute a representative panel of specimens containing a wide range of the respiratory pathogens (viruses and atypical bacteria) tested in the evaluated kits, 88 respiratory samples (30 from the University Hospital of Caen and 58 from the University Hospital of Saint-Etienne, France; one half being nasal swab and one half being respiratory secretions) were selected (by S.P., F.G., J.D. and A.V.) for the study. Viral and bacterial pathogens were routinely detected by immunofluorescence assay (respiratory syncytial viruses, influenza viruses, parainfluenza viruses, adenoviruses, and metapneumoviruses in Saint-Etienne) or molecular methods (home-brew method for influenza A
**Table 1.** Presentation of the 7 kits used in the study for detecting viruses (see text for correspondence between letters and commercial denominations).

| Gold standard | A | B | C | D | E | F |
|---------------|---|---|---|---|---|---|
| Influenza A viruses | Yes | Yes w diff A(H1N1) pdm09 | Yes | Yes | Yes w diff A(H1N1) pdm09 | Yes w diff (H1 and H3) | Yes |
| Other influenza viruses | B | B | B | B and C | B | B |
| Respiratory syncytial virus | Yes | Yes w diff | Yes w diff | Yes | Yes w diff | Yes | Yes w diff |
| Metapneumovirus | Yes | Yes | Yes | Yes | Yes | Yes |
| Parainfluenza virus | Yes | Yes w diff | Yes w diff | Yes w diff | Yes w diff | Yes w diff |
| Micoviruses (rhinovirus/enterovirus) | Yes | Yes | Yes w diff between rhinovirus A, B and C, and enterovirus | Yes w diff rhinovirus and enterovirus B | Yes | Yes w diff rhinovirus and coxsackie virus/echovirus |
| Coronavirus | Yes | Yes w diff between the 4 types | Yes (except HKU1) w diff between OC43 and 229E/NL63 | Yes (except HKU1) w diff between the 3 types | Detection of 229E only | Yes w diff between the 4 types | Yes w diff between the 4 types |
| Adenoviruses | Yes (A to G) | Yes | Yes | Yes | Yes | Yes | Yes (B and E only) |
| Bocavirus | Yes | Yes | Yes | Yes | Yes | Yes |
| Mycoplasma pneumoniae | Yes | Yes | Yes | No | No | No |
| Chlamydia pneumoniae | Yes | Yes | Yes | No | No | No |
| Bordetella pertussis | Yes | Yes | Yes | No | No | No |

Yes: group of pathogens detected; w diff: with differentiation between types. No: group of pathogens not detected.

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Table 2. List of pathogens that were considered to be present in the panel of the 88 specimens according to the combination of duplex PCR tests (Argene/bioMérieux) used as gold standard.

| Pathogens                        | Single infections | Co-infections with another pathogen | Co-infections with at least 2 other pathogens | Total     |
|----------------------------------|-------------------|-------------------------------------|-----------------------------------------------|-----------|
| Influenza A viruses              | 4                 | 3                                   | 0                                             | 7         |
| Influenza B viruses              | 0                 | 5                                   | 0                                             | 5         |
| Respiratory syncytial virus      | 3                 | 4                                   | 6                                             | 13        |
| Metapneumovirus                  | 0                 | 2                                   | 2                                             | 4         |
| Parainfluenza viruses\(^a\)      | 3                 | 4                                   | 3                                             | 10        |
| Rhinoviruses/enteroviruses       | 8                 | 5                                   | 10                                            | 23        |
| Coronaviruses\(^b\)              | 7                 | 5                                   | 13                                            | 25        |
| Adenoviruses                     | 2                 | 9                                   | 11                                            | 22        |
| Bocaviruses                      | 1                 | 4                                   | 7                                             | 12        |
| Mycoplasma pneumoniae            | 2                 | 4                                   | 4                                             | 10        |
| Chlamydophila pneumoniae         | 1                 | 1                                   | 2                                             | 4         |
| Bordetella pertussis             | 2                 | 4                                   | 3                                             | 9         |
| None                             | –                 | –                                   | –                                             | 11        |

\(^a\)type 1 \((n = 2)\), type 3 \((n = 3)\) and type 4 \((n = 5)\).
\(^b\)type NL63 \((n = 6)\), type OC43 \((n = 8)\), type 229E \((n = 1)\), type HKU1 \((n = 6)\) and untyped \((n = 4)\).

The respective sensitivity, specificity, accuracy and agreement (as evaluated by the Cohen kappa coefficient) of each tested kit were calculated by reference to the performances of the Argene/bioMérieux duplex tests used as gold standard.

Results

Characterization of the Panel and Overall Results

Following testing of the 88 samples with the reference method, the number of each pathogen considered to be present in the panel was as depicted in Table 2. Most pathogens, with the exception of influenza A virus, were frequently associated with at least another pathogen. The performances of each kit are summarized in Table 3 for viruses and in Table 4 for bacteria.

Sensitivity of the Tested Kits for Detecting Respiratory Pathogens

The overall sensitivity ranged from 56.25% to 91.67% for viruses (Table 3). Kits B and F exhibited the poorest sensitivity for most viral agents. The other tests were found sensitive for influenza viruses (except for kit E with influenza B virus), RSV, metapneumovirus and parainfluenza viruses, but less sensitive than the gold standard for rhinoviruses/enteroviruses, adenoviruses and bocavirus. The panel composition for coronaviruses (Table 2) explains the low sensitivity of kits B and D that detect only some types of these pathogens (Table 1). Regarding bacteria, the sensitivity of kits A and B was satisfying for Mycoplasma pneumoniae (80% for Chlamydia pneumoniae and close to 10% for Bordetella pertussis (Table 4).

Other Performances of the Tested Kits

The overall specificity of all the kits was excellent (>94% for all pathogens); the lowest specificity was observed for rhinoviruses/enteroviruses with kits C and E (84.4% and 83.3%, respectively). Globally, the accuracy was adequate, even if lower values were observed for rhinoviruses/enteroviruses and adenoviruses. The
Table 3. Performances of the 6 kits evaluated in this study for a panel of 9 viruses or groups of viruses with reference to the combination of duplex PCR tests (Argene/bioMérieux) used as gold standard.

| Pathogens tested | No. of positive specimens with the reference test | Performances | Evaluated kits |
|------------------|--------------------------------------------------|--------------|----------------|
|                  |                                                  | A            | B             | C             | D             | E             | F             |
|                  |                                                  | sensitivity %| specificity % | accuracy      | kappa coefficient | 95% confidence interval |
| Influenza A      | 7                                                | 100          | 57.14         | 100           | 100           | 100           | 57.14         |
|                  |                                                  | specificity %|              |              |               |               |               |
|                  |                                                  | 100          | 100.00        | 98.78         | 98.78         | 100           | 100           |
|                  |                                                  | accuracy     | 1.00          | 0.97          | 0.99          | 0.99          | 1.00          |
|                  |                                                  |              |               |               | 0.71 [0.40; 1] |               |               |
|                  |                                                  |              |               |               | 0.93 [0.79; 1] |               |               |
|                  |                                                  |              |               |               | 0.93 [0.79; 1] |               |               |
|                  |                                                  |              |               |               | 1 [1;1]       |               |               |
|                  |                                                  |              |               |               | 0.71 [0.40; 1] |               |               |
| Influenza B      | 5                                                | 80.00        | 60.00         | 100           | 100           | 60.00         | 60.00         |
|                  |                                                  | specificity %|              |              |               |               |               |
|                  |                                                  | 100          | 98.81         | 98.81         | 97.65         | 100           | 98.81         |
|                  |                                                  | accuracy     | 0.99          | 0.97          | 0.99          | 0.98          | 0.98          |
|                  |                                                  |              |               |               | 0.65 [0.28; 1]|               |               |
|                  |                                                  |              |               |               | 0.90 [0.72; 1]|               |               |
|                  |                                                  |              |               |               | 0.82 [0.58; 1]|               |               |
|                  |                                                  |              |               |               | 0.74 [0.39; 1]|               |               |
|                  |                                                  |              |               |               | 0.65 [0.28; 1]|               |               |
| RSV              | 13                                               | 92.31        | 92.31         | 100           | 100           | 92.31         | 69.23         |
|                  |                                                  | specificity %|              |              |               |               |               |
|                  |                                                  | 98.68        | 96.15         | 91.46         | 96.15         | 100           | 100           |
|                  |                                                  | accuracy     | 0.98          | 0.95          | 0.92          | 0.97          | 0.99          |
|                  |                                                  |              |               |               | 0.80 [0.56; 0.92]|               |               |
|                  |                                                  |              |               |               | 0.74 [0.56; 0.92]|               |               |
|                  |                                                  |              |               |               | 0.88[0.74; 1]|               |               |
|                  |                                                  |              |               |               | 0.95 [0.86; 1]|               |               |
|                  |                                                  |              |               |               | 0.79 [0.60; 0.99]|               |               |
| Meta pneumovirus | 4                                                | 100          | 75.00         | 100           | 100           | 100           | 75.00         |
|                  |                                                  | specificity %|              |              |               |               |               |
|                  |                                                  | 98.82        | 93.33         | 98.82         | 98.82         | 98.82         | 98.82         |
|                  |                                                  | accuracy     | 0.99          | 0.92          | 0.99          | 0.98          | 0.98          |
|                  |                                                  |              |               |               | 0.83 [0.67; 0.99]|               |               |
|                  |                                                  |              |               |               | 0.74 [0.56; 0.92]|               |               |
|                  |                                                  |              |               |               | 0.88 [0.66; 1]|               |               |
| Parainfluenza viruses | 10                  | 90.00        | 80.00         | 100           | 100           | 100           | 70.00         |
|                  |                                                  | specificity %|              |              |               |               |               |
|                  |                                                  | 97.50        | 97.50         | 92.86         | 97.50         | 97.50         | 100           |
|                  |                                                  | accuracy     | 0.97          | 0.95          | 0.93          | 0.98          | 0.98          |
|                  |                                                  |              |               |               | 0.80 [0.56; 0.99]|               |               |
|                  |                                                  |              |               |               | 0.73 [0.53; 0.93]|               |               |
|                  |                                                  |              |               |               | 0.90 [0.76; 1]|               |               |
|                  |                                                  |              |               |               | 0.81 [0.59; 1]|               |               |
| Rhinoviruses/enteroviruses | 23                  | 86.96        | 39.13         | 91.30         | 82.61         | 100           | 82.61         |
|                  |                                                  | specificity %|              |              |               |               |               |
|                  |                                                  | 91.55        | 100           | 84.42         | 90.28         | 83.33         | 94.20         |
|                  |                                                  | accuracy     | 0.90          | 0.84          | 0.84          | 0.88          | 0.85          |
|                  |                                                  |              |               |               | 0.75 [0.59; 0.90]|               |               |
|                  |                                                  |              |               |               | 0.49 [0.28; 0.70]|               |               |
|                  |                                                  |              |               |               | 0.64 [0.50; 0.78]|               |               |
|                  |                                                  |              |               |               | 0.69 [0.52; 0.86]|               |               |
|                  |                                                  |              |               |               | 0.68 [0.52; 0.83]|               |               |
|                  |                                                  |              |               |               | 0.77 [0.61; 0.92]|               |               |
| Coronaviruses    | 25                                               | 80.00        | 24.00 *       | 84.00         | 4.00 b        | 76.00         | 48.00         |
|                  |                                                  | specificity %|              |              |               |               |               |
|                  |                                                  | 98.44        | 100           | 98.44         | 100           | 96.92         | 100           |
|                  |                                                  | accuracy     | 0.93          | –             | 0.94          | –             | 0.91          |
|                  |                                                  |              |               |               |               |               | 0.85          |
### Table 3. Cont.

| Pathogens tested | No. of positive specimens with the reference test | Performances | Evaluated kits |
|------------------|--------------------------------------------------|--------------|----------------|
|                  |                                                  | A            | B             | C             | D             | E             | F             |
| Adenoviruses     | 22                                              | sensitivity %| 63.64         | 45.45         | 86.36         | 59.09         | 45.45         | 31.82         |
|                  |                                                  | specificity %| 100           | 100           | 92.96         | 100           | 100           | 100           |
|                  |                                                  | accuracy     | 0.91          | 0.86          | 0.91          | 0.90          | 0.86          | 0.83          |
|                  |                                                  | kappa coefficient [95% confidence interval] | 0.72 [0.55; 0.90] | 0.56 [0.35; 0.77] | 0.77 [0.61; 0.92] | 0.68 [0.50; 0.87] | 0.66 [0.36; 0.77] | 0.66 [0.19; 0.63] |
| Bocavirus        | 12                                              | sensitivity %| 50.00         | 25.00         | 75.00         | 58.33         | 33.33         | 16.67         |
|                  |                                                  | specificity %| 100           | 100           | 97.44         | 98.70         | 100           | 100           |
|                  |                                                  | accuracy     | 0.93          | 0.90          | 0.94          | 0.93          | 0.91          | 0.89          |
|                  |                                                  | kappa coefficient [95% confidence interval] | 0.63 [0.37; 0.90] | 0.37 [0.06; 0.67] | 0.75 [0.54; 0.96] | 0.66 [0.42; 0.91] | 0.46 [0.16; 0.76] | 0.26 [0; 0.55] |
| Overall (except coronaviruses) | 96                                              | sensitivity %| 79.17         | 54.17         | 91.67         | 81.25         | 76.04         | 56.25         |
|                  |                                                  | specificity %| 98.41         | 98.16         | 94.32         | 97.28         | 97.46         | 99.08         |
|                  |                                                  | accuracy     | 0.96          | 0.92          | 0.94          | 0.95          | 0.94          | 0.93          |
|                  |                                                  | kappa coefficient [95% confidence interval] | 0.81 [0.75; 0.88] | 0.61 [0.51; 0.70] | 0.77 [0.70; 0.83] | 0.79 [0.72; 0.86] | 0.76 [0.69; 0.83] | 0.66 [0.58; 0.73] |

*Kit having no probe for the detection of HKU1 coronavirus.

*Kit having a probe for detecting 229E coronavirus only.

*All the tested kits did not detect all the coronavirus types (see notes a and b).
Cohen kappa coefficient, which reflects the agreement with the gold standard, was globally strong for viruses (≥0.60) (Table 3) and moderate for bacteria (0.41–0.60) (Table 4).

**Practicability**

The criteria evaluated for the technical and workflow characteristics of each kit are depicted in Table 5. Ten to 22 samples were analyzed in one run. The extraction step was controlled by the addition of an internal control in 3 of the 7 kits. Ten to 70 µl of extract were needed for analysis. After the extraction step, the hands-on time varied from 50 min to 2h15 depending on the number of reaction tube opening, the availability of ready-to-use reagents and the number of mastermix. The signal interpretation was driven by using dedicated software in only three kits. The run duration varied from 2h30 to 9 h.

**Discussion**

To our knowledge, this study is the first that compared 6 commercialized multiplex PCR techniques for the detection of

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**Table 4.** Performances of the 2 kits evaluated in this study for a panel of 3 atypical bacteria with reference to the combination of duplex PCR tests (Argene/bioMérieux) used as gold standard.

| Pathogens tested | No. of positive specimens with the reference test | Performances | Evaluated kits |
|------------------|--------------------------------------------------|--------------|----------------|
|                  |                                                  |              | A              |
|                  |                                                  |              | B              |
| Mycoplasma pneumoniae | 10                                                | sensitivity % | 70.00          |
|                   |                                                  | specificity %| 100            |
|                   |                                                  | accuracy     | 0.97           |
|                   |                                                  | kappa coefficient [95% confidence interval] | 0.81 [0.59; 1] |
| Chlamyphilia pneumoniae | 4                                                 | sensitivity % | 50.00          |
|                     |                                                  | specificity %| 100            |
|                     |                                                  | accuracy     | 0.98           |
|                     |                                                  | kappa coefficient [95% confidence interval] | 0.66 [0.21; 1] |
| Bordetella pertussis | 9                                                 | sensitivity % | 11.11          |
|                    |                                                  | specificity %| 100            |
|                    |                                                  | accuracy     | 0.91           |
|                    |                                                  | kappa coefficient [95% confidence interval] | 0.18 [0; 0.50] |
| Overall            | 23                                               | sensitivity % | 43.48          |
|                   |                                                  | specificity %| 100            |
|                   |                                                  | accuracy     | 0.95           |
|                   |                                                  | kappa coefficient [95% confidence interval] | 0.58 [0.38; 0.79] |

**Table 5.** Practicability of each kit using the NUCLISENS easyMAG as extraction instrument (see text for correspondence between letters and commercial denominations).

| Gold standard | A | B | C | D | E | F |
|---------------|---|---|---|---|---|---|
| Throughput (number of tested specimen/run) | 10 | 22 | 13 | 22 | 22 | 22 |
| Hands on time | 50 min | 2h15 | 2h15 | 1h45 | 2 h | 1h30 | 1h30 |
| Run duration | 2h30 | 7 h | 7 h | 5h30 | 9 h | 4h30 | 4h30 |
| Number of reaction tubes opening | 0 | 2 | 1 | 2 | 1 | 1 | 3 |
| Ready to use reagents | yes | no | no | no | yes | no | no |
| Addition of internal control at the extraction step | no | yes | no | no | no | yes | yes |
| Amplification of a cellular gene control | yes | no | yes | yes | no | no | no |
| Volume of extracted sample | 100 µl | 10 µl | 33 µl | 11 µl | 10 µl | 10 µl | 10 µl |
| Number of mixtures | 7 | 2 | 4 | 3 | 2 then 1 | 1 | 1 |
| Software for results interpretation | no | no | yes | no | yes | yes | no |
| Access to raw data | yes | yes | yes | yes | yes | yes | yes |

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respiratory pathogens. Five of these kits (A, B, D, E and F) had been previously evaluated versus conventional techniques [27–31], home-brew PCR [29], [31–33], or other commercial multiplex kits [27–31], [33–34], [36–41]. However, none of these studies compared more than 4 commercialized techniques simultaneously. It is noteworthy that the 6 kits tested in this study do not constitute an exhaustive panel of the techniques presently commercialized on the world market but are representative of those available in Europe at the beginning of 2011.

The present comparison includes two multiplex techniques (A and B) that were able to detect respiratory viruses together with atypical bacteria. The molecular diagnosis of these bacteria is most often performed by using home-brew methods and few commercial techniques have been evaluated comparatively [20–23]. The simultaneous detection of atypical bacteria and viruses represents a great advantage in terms of clinical management. Indeed, in case of positive result for an atypical bacterium, a rapid treatment with an adapted antimicrobial drug can be proposed [22]; by contrast, if this information is lacking, there is a risk for prescribing no treatment, especially if the specimen is also found positive for one or several viruses, a rather common situation with the present panel. In the opposite case, the absence of atypical bacteria (together with the negativity of conventional bacterial cultures) would allow to spare useless antibiotic treatment and consequently the emergence of bacterial resistance [7].

This study exhibits a few limitations. No gold standard is available for each of the 12 pathogens tested in this study. Even if molecular techniques are globally considered as the most sensitive, it is difficult to identify a precise method as the reference for a given pathogen or family of pathogens. In addition, the sensitivity may vary when the test is adapted to the whole family (adenoviruses, rhinoviruses/enteroviruses, parainfluenza viruses, coronaviruses…) or specific for a single type. Despite this difficulty, the Argene/bioMérieux strategy, which consists in the combination of duplex real time PCR methods, was taken as gold standard with reference to conventional techniques [34], [44–46]. These performances are globally satisfactory, at least for those that are still commercially-available in 2013. On the basis of the present evaluation, the spectrum of detected pathogens (with an advantage to the techniques detecting also atypical bacteria), the technology used for PCR product revelation and the laboratory organization (including the use of an internal control and, if possible, of a cellular control) are advocated for evaluating the cost-benefit of these techniques in the clinical management of respiratory infection.

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Author Contributions
Conceived and designed the experiments: SP FG JL AV BP. Performed the experiments: SP ML JD FG. Analyzed the data: SP ML FG JL AV BP. Contributed reagents/materials/analysis tools: SP JD FG POV AV. Wrote the paper: SP BP.

References
1. Glezen P, Denny FW (1973) Epidemiology of acute lower respiratory disease in children. N Engl J Med 288: 498–505. doi:10.1056/NEJM197303082881005.
2. Pavía AT (2011) Viral infections of the lower respiratory tract: old viruses, new viruses, and the role of diagnosis. Clin Infect Dis 52 Suppl 4: S284–289. doi:10.1093/cid/cir043.
3. Gonzales R, Malone DC, Maselli JH, Sande MA (2001) Excessive antibiotic use for acute respiratory infections in the United States. Clin Infect Dis 33: 737–762. doi:10.1093/cid/33.4.737.
4. Schneif N, Resche-Rigon M, Chaillon A, Scemla A, Gras G, et al. (2011) High burden of non-influenza viruses in influenza-like illness in the early weeks of
Infections during three consecutive outbreaks in Normandy, France. J Med Virol 83: 517–524. doi:10.1002/jmv.22002.

26. Dina, J., Nguyen E, Gouarin S, Petjane J, Parietien J-J, et al. (2009) Development of duplex real-time PCR for detection of two DNA respiratory viruses. J Med Virol 81: 119–125. doi:10.1002/jmv.20856.

27. Bahade NL, Mead P, Stiles J, Brennan C, Li H, et al. (2012) Comparison of the Luminex xTAG RVP Fast assay and the Idaho Technology FilmArray RP assay for detection of respiratory viruses in pediatric patients at a cancer hospital. J Clin Microbiol 50: 2282–2289. doi:10.1128/JCM.00616-11.

28. Balula-Lisat JM, LaRue H, Kelly C, Igali L, Pancholi P (2011) Evaluation of commercial ResPlex II v2.0, MultiCode-PLX, and xTAG respiratory viral panel for the diagnosis of respiratory viral infections in adults. J Clin Virol 50: 42–45. doi:10.1016/j.jcv.2010.09.032.

29. Bruinestein van Coppenratt LES, Swannick CMA, Van Zee AA, Nijhuis RHT, Schirm J, et al. (2010) Comparison of two commercial molecular assays for simultaneous detection of respiratory viruses in clinical samples using two automatic electrophoresis detection systems. J Virol Methods 169: 180–190. doi:10.1016/j.jviromet.2010.07.032.

30. Gharabaghi F, Haswan A, Dreeso SJ, Richardson SE (2011) Evaluation of multiple commercial molecular and conventional diagnostic assays for the detection of respiratory viruses in children. Clin Microbiol Infect 17: 1900–1906. doi:10.1111/j.1469-0691.2011.03529.x.

31. Jokela P, Pipipainen I, Mannonen L, Auvinen E, Lappalainen M (2012) Performance of the Luminex xTAG Respiratory Viral Panel Fast in a clinical laboratory setting. J Virol Methods 182: 86–86. doi:10.1016/j.jviromet.2012.02.015.

32. Bibby DF, Elsmaney I, Breuer J, Clark DA (2011) Comparative evaluation of the Seegene Seeplex RV15 and real-time PCR for respiratory virus detection. J Med Virol 83: 1469–1475. doi:10.1002/jmv.22125.

33. Hayden RT, Gu Z, Rodriguez A, Tannahia L, Ying G, et al. (2012) Comparison of two broadly multiplexed RT-PCR systems for viral detection in clinical respiratory tract specimens from immunocompromised children. J Clin Virol 53: 308–313. doi:10.1016/j.jcv.2011.12.020.

34. Li H, McCormae MA, Estes RW, Seferes SE, Dare RK, et al. (2007) Simultaneous detection and high-throughput identification of a panel of RNA viruses causing respiratory tract infections. J Virol Methods 145: 2105–2109. doi:10.1016/j.jviromet.2009.07.004.

35. Wang W, Ken P, Sheng J, Marcy S, Yan H, et al. (2009) Simultaneous detection of respiratory viruses in children with acute respiratory infection using two different multiplex reverse transcription-PCR assays. J Virol Methods 162: 40–45. doi:10.1016/j.jviromet.2009.07.004.

36. Looms K, Van Loon AM, Coenjaerts T, Van Aarle Y, Goossens H, et al. (2012) Performance of different multiplex and multiplex nucleic acid amplification tests on a multipathogen external quality assessment panel. J Clin Microbiol 50: 977–987. doi:10.1128/JCM.00210-07.

37. Rand KH, Ramsperaud S, Houch HJ (2011) Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. J Clin Microbiol 49: 2449–2453. doi:10.1128/JCM.00522-10.

38. Raymaekers M, De Rijke B, Paoli I, Van den Abeele AM, Cartuyvels Y (2011) Timely diagnosis of respiratory tract infections: evaluation of the performance of the Respifinder assay compared to the xTAG respiratory viral panel assay. J Clin Virol 52: 314–316. doi:10.1016/j.jcv.2011.08.017.

39. Dabisch-Rute H, Vollner T, Adams O, Knabbe C, Dreier J (2012) Comparison of three multiplex PCR assays for the detection of respiratory viral infections: evaluation of xTAG respiratory virus panel fast assay, RespFinder 19 assay and RespFinder SMART 22 assay. BMC Infect Dis 12: 163. doi:10.1186/1471-2334-12-163.

40. Popowitch EB, O’Neill SS, Miller MB (2013) Comparison of four multiplex assays for the detection of respiratory viruses: Biofire FilmArray RP, Genmark eSensor RVP, Luminex xTAG RVPv1 and Luminex xTAG RVP FAST. J Clin Microbiol, in press. doi:10.1128/JCM.03688-12.

41. Forman MS, Advani S, Newman C, Gaydos CA, Milstone AM, et al. (2012) Diagnostic performance of two highly multiplexed respiratory virus assays in a pediatric cohort. J Clin Virol 55: 160–167. doi:10.1016/j.jcv.2012.06.019.

42. Havala H, Wohlert KC, Simonetti P (2010) Par cochoviruses in children: understanding a new infection. Curr Opin Infect Dis 23: 224–230.

43. Piralla A, Furione M, Rovida F, Marchi A, Stronati M, et al. (2012) Human Pneumovir DNA array. J Med Virol 83: 150–155. doi:10.1002/jmv.22092.

44. Lui G, Ip M, Lee N, Rainer TH, Man SY, et al. (2011) Development of a non-commercialised, expanded triplex PCR diagnostic system for simultaneous detection of respiratory viruses in clinical specimens by multiplex PCR. J Clin Microbiol 43: 3247–3254. doi:10.1128/JCM.00473-11.

45. Loons K, Beck T, Uusi D, Ovrelid M, Silkevans P, et al. (2008) Evaluation of different nucleic acid amplification techniques for the detection of M. pneumoniae, C. pneumoniae and Legionella spp. in respiratory specimens from patients with community-acquired pneumonia. J Microbiol Methods 73: 257–262. doi:10.1016/j.mimet.2008.02.010.

46. Liu G, Ip M, Lee N, Rainer TH, Man SY, et al. (2009) Role of “atypical pathogens” among adult hospitalized patients with community-acquired pneumonia. Respir Res 10: 1085–1100. doi:10.1186/1465-9921-10-1085.

47. Mahony JB, Black F, McElarney I, Breuer J, Clark DA (2011) Comparative evaluation of the FilmArray Respiratory Panel and Prodesse real-time PCR assays for detection of respiratory viral pathogens. J Clin Microbiol 49: 4083–4088. doi:10.1128/JCM.05010-11.

48. Loffeldhozl MJ, Pong DL, Olofsson S, Lindh M, Andersson L-M (2011) Access to a multiplex PCR system for detection of respiratory viral infections and coinfections in patients with acute respiratory illness. J Med Virol 83: 1498–1504. doi:10.1002/jmv.20725.

49. Mahony JB, Petrich A, Smieja M, Van Aarle Y, Goossens H, et al. (2012) Simultaneous detection and high-throughput identification of a panel of RNA viruses causing respiratory tract infections. J Virol Methods 145: 2105–2109. doi:10.1016/j.jviromet.2009.07.004.

50. Piralla A, Furione M, Rovida F, Marchi A, Stronati M, et al. (2012) Human Pneumovir DNA array. J Med Virol 83: 150–155. doi:10.1002/jmv.22092.