Laboratory Impact of Rapid Molecular Tests used for the Detection of Respiratory Pathogens

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SUMMARY

Background: With outbreaks of new respiratory viruses such as the severe acute respiratory syndrome coronavirus and swine-origin influenza A/H1N1, the nucleic acid-based amplification test was introduced to identify causative agents. Multiplex PCR, which can simultaneously detect various respiratory pathogens, is currently used worldwide. Recently, a new type of multiplexed molecular test using a fully automated workflow system was developed, which was also adapted to our laboratory. In this study, we assessed improvements in laboratory practice brought about by the implementation of the rapid test for the detection of respiratory viruses.

Methods: We investigated the number of routine and rapid tests conducted as well as the change in monthly test frequency of the routine test. We also analyzed the waiting time, turnaround time, and lead time for the routine and rapid tests. The Anyplex II RV16 detection kit (Seegene, Seoul, Korea) and Filmarray Respiratory Panel (BioFire Diagnostics, Inc., Salt Lake City, UT, USA) were used for the routine and rapid tests, respectively.

Results: Compared to the routine test, the rapid test significantly (p < 0.01) decreased the mean waiting time (1 hour 46 minutes), turnaround time (1 hour 45 minutes), and lead time (3 hours 32 minutes). After the implementation of the rapid test, the number of routine tests conducted was reduced over the 5-month period, from 13 times a month to 3 times a month.

Conclusions: The implementation of the rapid test for the detection of respiratory viruses improved the diagnostic efficiency of the laboratory and greatly reduced lead time.


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INTRODUCTION

Respiratory infection is a major cause of infant hospitalizations and deaths worldwide. The major causative agents of respiratory diseases include influenza virus, parainfluenza virus (PIV), respiratory syncytial virus (RSV), adenovirus (AdV), and human rhinovirus (HRV). However, recently, new viruses including severe acute respiratory syndrome coronavirus (SARS-CoV [2003]), swine-origin influenza A (H1N1 pdm 2009), and Middle East respiratory syndrome coronavirus (MERS-CoV [2012]) have raised problems worldwide [1].
There is no significant difference between the symptoms of respiratory tract infection caused by different causative agents, and most treatments are aimed toward relieving symptoms rather than eliminating the infection [2,3]. Therefore, diagnostic tests are typically not performed for most cases. However, the appearance of new viruses has created a need for developing diagnostic methods to identify the viruses responsible for the infections [1,4]. For example, in the case of H1N1 influenza, it became critical to rapidly isolate patients and administer appropriate antiviral drugs; therefore, PCR-based viral detection methods came to be used. Now, rapid diagnostic tests for influenza or multiplex PCR tests that simultaneously detect various respiratory viruses are commonly used worldwide [1,4,5]. The methods used for isolation and treatment may differ depending on the type of causative pathogen, but initial isolation and treatment are important in cases of respiratory tract infections that cause serious symptoms. For the diagnosis and treatment are important in cases of respiratory tract infection caused by different agents, and most treatments are aimed toward relieving symptoms rather than eliminating the infection [2,3]. Therefore, diagnostic tests are typically not performed for most cases. However, the appearance of new viruses has created a need for developing diagnostic methods to identify the viruses responsible for the infections [1,4]. For example, in the case of H1N1 influenza, it became critical to rapidly isolate patients and administer appropriate antiviral drugs; therefore, PCR-based viral detection methods came to be used. Now, rapid diagnostic tests for influenza or multiplex PCR tests that simultaneously detect various respiratory viruses are commonly used worldwide [1,4,5]. The methods used for isolation and treatment may differ depending on the type of causative pathogen, but initial isolation and treatment are important in cases of respiratory tract infections that cause serious symptoms.

To overcome this problem, recently, a rapid test that can detect 17 viruses and 3 bacteria with a turn-around-time (TAT) of 1 hour was developed [6]. The sensitivity and specificity of this rapid test were similar to that of the existing diagnostic test. An additional advantage of the rapid test was that it has the simplest workflow. It was therefore introduced as an emergency test. In the present study, we aimed to study the effects of the implementation of the above-mentioned rapid test for the detection of respiratory viruses on laboratory practices.

**MATERIALS AND METHODS**

Analyses were performed for results of the PCR tests (routine and rapid tests) for respiratory viruses conducted at Yeungnam University Medical Center from 2014 to July 2017.

1) To analyze the laboratory status, the number of cases in each month and test frequency of monthly routine PCR tests for respiratory viruses conducted from January 2014 to December 2016 were calculated; the distribution of the requesting departments was also investigated.

2) To minimize seasonal variations, the virus detection rate was investigated based on the results of the routine tests conducted from November 2015 to July 2016 and the results of the rapid tests conducted from November 2016 to July 2017.

3) Positive rates of influenza A virus (INF A), influenza B virus (INF B), RSV, metapneumovirus (MPV), AdV, coronavirus (CoV), human enterovirus (HEV), PIV, and HEV, which are commonly detected in the routine and the rapid tests, were determined.

4) Since the implementation of the rapid test from November 2016 to March 2017, the number of routine and rapid tests conducted and the changes in the frequency of the use of the routine test were determined for each month. In addition, the number of tests conducted during regular hours (weekdays from 08:30 to 17:30) and night hours/holidays (other than regular hours) were investigated based on the time when the test was conducted.

5) The waiting time (from prescription to submission of specimen to the laboratory), TAT (from submission of specimen to final result), and lead time (from prescription to final result) of the routine test (November 2015 to July 2016) and rapid test (November 2016 to July 2017) were analyzed.

**Routine test**

The routine multiplex PCR tests for detecting respiratory viruses were performed using Anyplex II RV16 detection kit (RV16; Seegene, Seoul, Korea). RV16 could detect 16 viruses including INF A (subtypes H1, H2, H3, H5, H6, H7, H9, H10, and H11), INF B, RSV A and RSV B, AdV (serotypes A to F), MPV, CoV 229E, CoV NL63, CoV OC43/HKU1, PIV 1 to 4, HRV A to C, HEV, and bocavirus 1 to 4.

**Rapid test**

A Filmarray Respiratory Panel (RP; BioFire Diagnostics, Inc., Salt Lake City, UT, USA) was used for the rapid test, which can target 20 viruses and bacteria including INF A (including subtypes H1N1, H3N2 and the 2009-H1N1), INF B, RSV, AdV, MPV, HRV, HEV, CoV (HKU1, NL63, 229E, and OC43), PIV-1, PIV-2, PIV-3, PIV-4, *Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*.

**Statistical analyses**

Student’s *t*-test was applied to compare the waiting time, TAT, and lead time between the routine and rapid tests.

**RESULTS**

**Laboratory status**

From January 2014 to December 2016, the mean number of routine tests conducted was 204.1 times per month, and the mean number of tests conducted in February and March during the 3-year period was 380.7 and 360, respectively. The highest number of tests conducted was in February 2014 (535 tests; Figure 1). The mean test frequency was 15.7 times per month, and test frequency increased to 22.7 times in February and 26.3 times in March; these numbers were higher than those for the rest of the year. In particular, tests were run 33 times in February 2016 and 41 times in March 2016 (Figure 2). The highest requests for the routine test were from emergency medicine (44.3%), pediatrics (30.6%), internal medicine (22%), and other depart-
Table 1. Waiting time, TAT, and lead time of rapid and routine tests.

<table>
<thead>
<tr>
<th></th>
<th>Routine test</th>
<th></th>
<th>Rapid test</th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Waiting time</td>
<td>12 hours 54 minutes</td>
<td>13 hours 39 minutes</td>
<td>1 hour 46 minutes</td>
<td>2 hours 46 minutes</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>TAT</td>
<td>19 hours 51 minutes</td>
<td>16 hours 23 minutes</td>
<td>1 hour 45 minutes</td>
<td>2 hours 21 minutes</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Lead time</td>
<td>32 hours 45 minutes</td>
<td>18 hours 40 minutes</td>
<td>3 hours 32 minutes</td>
<td>3 hours 36 minutes</td>
<td>&lt; 0.01</td>
</tr>
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Figure 1. Number of cases in each month (2014 - 2016).

Figure 2. Monthly test frequency (2014 - 2016).
Comparison of detection rate by virus type
Both the rapid and routine tests showed the highest detection rates for HRV (23.3% and 28.4%, respectively), and the lowest for FluB and HEV (5.0% and 2.03%, respectively; Figure 3). The monthly trends of the detection rates for each virus are shown in Figure 4.

Changes in waiting time, TAT, and lead time with the use of the rapid test
For the routine tests conducted from November 2015 to July 2016, the average waiting time, TAT, and lead time were 12 hours 54 minutes, 19 hours 51 minutes, and 32 hours 45 minutes, respectively. For the rapid tests conducted from November 2016 to July 2017, the mean waiting time (1 hour 46 minutes), TAT (1 hour 45 minutes), and lead time (3 hours 32 minutes) were significantly lower than those of the routine tests (p < 0.01) (Table 1).

Trends for number of tests conducted and distribution of test time after introduction of the rapid test
Since the introduction of the rapid test in November 2016, the numbers of requests for the rapid and routine tests changed. The number of routine tests conducted was reduced to 3 times in March 2017 from 13 times in November 2016 (Figure 5). Of the total number of tests conducted, 36.9% of the tests were conducted during the regular hours, whereas 63.1% of the tests were conducted during the evenings or holidays.

DISCUSSION
Although PCR was widely used for the detection of respiratory viruses in 2009 due to the outbreak of H1N1 influenza [7], the results of the PCR tests could not immediately lead to isolation or treatment. Due to batch operation, reviews of the results and follow-up actions were performed only after initial isolation or treatment. Moreover, because the PCR test is generally divided into three steps - nucleic acid extraction, amplification, and detection - the lead time as well as risk of contamination between the steps is higher. While a multiplex PCR method that can simultaneously perform nucleic acid extraction and amplification is commonly used, the PCR test cannot be used as an emergency test as it takes a long time to yield results. An immunochromatographic test is used as a rapid test to detect INF and can directly detect viral antigens [8]. Recently, its detection sensitivity has also been improved by using fluorescence or directly amplifying nucleic acids [9]. However, such an immunochromatographic test method is also not perfectly appropriate for use as an emergency test for screening because of its low negative predictive value attributable to its lower sensitivity compared to that of the diagnostic PCR test. This means that even if the test result is negative, a diagnostic PCR test result is required for confirmation. Due to this limitation of the immunochromatographic test method, it has little effect on treatment and isolation and is therefore less practical. The rapid test that we adopted is appropriate for use as a 24-hours rapid test because it can complete nucleic acid
Figure 4. Monthly trends in the detection rates for influenza A (A), influenza B (B), respiratory syncytial virus (C), metapneumovirus (D), adenovirus (E), coronavirus (F), human enterovirus (G), parainfluenza virus (H), and human rhinovirus (I) of routine (red line) and rapid (blue line) tests.
Figure 5. Trends for number of tests conducted and distribution of test time after introduction of the rapid test.

extraction, amplification, and detection rapidly within 1 hour, without the need for trained personnel. Furthermore, its sensitivity and specificity are equivalent to those of the existing diagnostic test [6]. This rapid test can also simultaneously detect various respiratory pathogens and is therefore believed to be capable of completely replacing the routine test.

Respiratory tract infections are influenced by seasons, and we identified that the number of tests and the test frequency increased during the winter and early spring (Figure 1, 2). Such rapid and temporary increases in the number of tests is a factor that makes laboratory operation unstable. In particular, when the routine test is run in batch operation, a sudden increase in the number of tests also increases the frequency of tests, which affects other tests that are regularly performed by the testers. Although a flexible arrangement of personnel and test equipment can be a solution, it is difficult to apply in reality. Therefore, we introduced a rapid test that can be run 24 hours (any time of the day), which would greatly reduce the frequency of the routine test. Now, the rapid test has completely replaced the routine test, which used to be performed 3 - 4 times a week requiring 4 - 5 hours for each run. In our laboratory, 36.9% of the rapid tests are conducted during regular hours, and the rest of the tests are performed during the evening or holiday hours; the time of conducting the routine tests is thus dispersed. The test does not require any special skill or training due to its simple workflow, and it only takes minutes to prepare a specimen. In addition, the target TAT was set to 4 hours to allow enough time for other basic tests to be conducted with priority and for a rapid respiratory test to be done during the remaining hours. This has greatly reduced the TAT compared to that of the existing test, while not affecting the regular tasks in both regular and emergency laboratories.

As such, if a batch test is converted to the 24-hours rapid test that allows random access, the waiting time, TAT, and lead time will become shorter. In the routine test, the mean waiting time from prescription to submission of specimen to the laboratory was 12 hours 54 minutes (Table 1), which is too long to guarantee maintenance of the original condition of the specimen submitted to the laboratory; thus, the laboratory has to take the risk of change in specimen quality for the test. A reduced mean lead time (from prescription to test report) from 33 hours 45 minutes to 3 hours 32 minutes is therefore of great significance from a clinical perspective. In the case of the routine test, the long period necessitates a general mode of treatment. On the other hand, with the rapid test, the mode of treatment can be decided based on the test result, which will greatly improve the choice of treatment. In addition, a prompt and appropriate treatment will affect the duration of hospitalization. A rapid isolation might also improve overall infection control in hospitals. However, the effect of the use of the rapid test on the duration of hospitalization, use of antibiotics, and clinical effects related to infection control in hospitals need to be studied in further detail; these experiments are being currently conducted as part of another study.

CONCLUSION

The implementation of the rapid test for the detection of respiratory viruses improved the laboratory work system and greatly reduced lead time.
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Declaration of Interest:
The authors declare that they have no conflict of interest related to the publication of this manuscript.

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