Utility of a Sputum Antigen Detection Test in Pneumococcal Pneumonia and Lower Respiratory Infectious Disease in Adults

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Abstract

Objective To compare the utility of Gram staining, a urinary antigen detection kit and a sputum antigen detection kit were examined for the rapid and early detection of pneumococcal pneumonia and lower respiratory infectious diseases.

Methods A newly developed sputum pneumococcal antigen detection kit (RAPIRUN), Gram staining, and urinary antigen detection kit (BinaxNOW) were comparatively evaluated for their ability to detect *Streptococcus pneumoniae* in patients with pneumonia or lower respiratory tract infection. Sputum culture results were used as a standard for comparison. Furthermore, the pneumococcus-positive rates in culture and rapid tests were compared using polymerase chain reaction (PCR) as a reference.

Results Of the 169 patients studied, 54 (32.0%) tested positive for *S. pneumoniae* in culture. *S. pneumoniae* detection sensitivities for Gram staining, RAPIRUN, and BinaxNOW were 75.9%, 90.7%, and 53.7%, respectively; thus, RAPIRUN had a significantly higher sensitivity than BinaxNOW ($p < 0.001$). For patients with $\geq 10^5$ copies/ μg of pneumococcal surface protein A DNA PCR analysis, the detection rates of culture, Gram staining, and RAPIRUN were 85.2%, 72.1%, and 82.0%, respectively, however, the detection rate of BinaxNOW was only 47.5%. Comparisons among 45 patients with culture-positive pneumococcal pneumonia revealed that RAPIRUN had a significantly higher detection rate than BinaxNOW in the mild cases ($p < 0.006$), regardless of the number of days from onset ($p < 0.03$).

Conclusion RAPIRUN is a rapid testing kit that detects *S. pneumoniae* in sputum with a high sensitivity and specificity. It is a particularly more useful detection kit than BinaxNOW for early and mild community-acquired pneumonia in pre-treatment patients whose sputum specimens can be obtained.

Key words: *Streptococcus pneumoniae*, sputum antigen, urinary antigen, rapid diagnostic test, Gram staining, acute lower respiratory tract infection

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Introduction

Streptococcus pneumoniae is the primary causative agent of pneumonia in Japan, and pneumococcal pneumonia is well-recognized as being potentially severe with a high mortality (1-4). Although pneumococcal 23-valent vaccination of the elderly has been reported to decrease pneumonia prevalence (5, 6), the rapid detection of *S. pneumoniae* in the early phase of the disease and early treatment are still important clinical measures that improve patient outcomes.

The sensitivity and specificity of conventional Gram staining of sputum have been shown to be affected by the skill of the technician (7). In a meta-analysis of adult community-acquired pneumonia (CAP), a test that detects urinary pneumococcal antigen (BinaxNOW® *S. pneumoniae*; BinaxNOW, Alere Scarborough, Scarborough, USA) has been reported to increase the diagnostic yield of *S. pneumoniae* by 11.4% compared with blood and sputum culture tests (8). BinaxNOW *S. pneumoniae* has been reported to have played a positive role in increasing the diagnostic rate of pneumococcal pneumonia (1). In contrast, several false-negative cases have been reported in urinary pneumococcal antigen tests. Among 171 CAP patients who tested negative in the urinary pneumococcal antigen test, 28 (16.4%) tested positive for *S. pneumoniae* by sputum culture and/or Gram staining (9). Furthermore, among patients with a *S. pneumoniae*-positive sputum culture, 44.4% (10) or 76.5% (11) tested negative in the urinary antigen test.

Recently, RAPIRUN® *S. pneumoniae* (hereafter referred to as RAPIRUN, Otsuka Pharmaceutical, Tokyo, Japan), a novel rapid diagnostic kit that visually detects a cell wall antigen common to all serotypes of *S. pneumoniae* (12) within 25 minutes by immunochromatography, has been developed. We investigated its clinical performance using the sputum culture test as a standard and reported that RAPIRUN *S. pneumoniae* detects *S. pneumoniae* with a higher sensitivity compared to BinaxNOW (10, 13, 14). However, the results of *S. pneumoniae* detection by various rapid tests have not yet been fully evaluated in comparison with those of polymerase chain reaction (PCR)-based detection methods.

In the present study, we comparatively investigated the performance of three rapid diagnostic tests using the sputum culture method as a standard in antibiotic-naïve patients (at least 15 years of age) with pneumonia or a lower respiratory tract infection. We also compared the *S. pneumoniae*-positive rates of the culture method and the various rapid tests using PCR as a reference.

Materials and Methods

This study was conducted at 14 medical institutions nationally from March 2006 to March 2007. The study was approved by the institutional review board of each institution, and written informed consent was obtained from each

subject after the study was carefully explained.

Study population

In patients who were at least 15 years of age and were diagnosed with pneumonia or a lower respiratory tract infection at the participating medical institutions, sputum specimens could be collected from 246 enrolled cases. Of the 246 patients, 169 antibiotic-naïve patients were analyzed.

CAP was diagnosed according to the Guidelines for the Management of Community-Acquired Pneumonia in Adults of the Japanese Respiratory Society (15). The severity of CAP was rated according to the A-DROP system, described in the abovementioned guidelines, which is a five-point scoring system based on age, dehydration, dyspnea, impaired consciousness, and a decrease in blood pressure. The number of days from onset was calculated considering the actual day of onset as zero.

Sample collection and microbiological investigations

Sputum and urine specimens were collected from the patients on the same day. All sputum samples were subjected to conventional Gram staining and then were transferred to the microbiology laboratory at Hokusho Central Hospital for expert evaluation as reported previously (13). In brief, sputum was used in Gram staining, the pneumococcal antigen detection test (RAPIRUN® *S. pneumoniae*; RAPIRUN), a simple quantitative culture test, and pneumococcal surface protein A (PspA) DNA quantification using real-time PCR. Urine samples were tested using the urinary antigen detection kit (BinaxNOW® *S. pneumoniae*; BinaxNOW).

In addition, post-culture bacterial counts were analyzed visually from the culture plate and graded on the following five-point scale: 3+, 2+, 1+, ±, and -. The relationships between the bacterial counts and the scale are approximately as follows: 1+ is equivalent to 10^3 - 10^5 CFU/mL, 2+ is 10^4 - 10^6 CFU/mL, and 3+ is 10^5 - 10^7 CFU/mL (15). PspA DNA quantification by PCR was performed at Kitasato-Otsuka Biomedical Assay Laboratories. (Sagamihara, Japan), as reported previously (13), and *S. pneumoniae* counts were expressed as the number of copies per 1 µg of DNA. *S. pneumoniae* detection sensitivity by this method was 4×10 copies/µg of DNA. We compared the detection rates of four methods based on the PCR results using two cutoff values, $\geq 10^5$ and $\geq 10^7$ copies/µg of DNA, according to the previous reports as follows: $\geq 10^5$ colony-forming units (CFU)/mL (16-18) and $\geq 10^7$ CFU/mL (19) by the quantitative culture method are used to confirm *S. pneumoniae* detected in the sputum specimen as a significant pathogen. The ratio of copies/µg of DNA by the PCR measurement system used in this study to the CFU/mL value in the quantitative culture method was almost 1:1 (13).

The quality of sputum specimens used in the tests was examined by microscopic observation of the Gram-stained smear samples at 100× magnification and was analyzed according to the classification method reported by Geckler et

al. (20), which is based on the numbers of buccal squamous epithelial cells and white blood cells.

Statistical analysis

The results of non-negative cases in culture were considered to be culture test-positive. The sensitivity, specificity, and concordance rate of the various rapid tests were determined using the culture method as the standard. The PCR-based PspA copy number was log transformed, and inter-group comparisons were performed using the Mann-Whitney U test or Dunn test. We compared the proportions of qualitative variables using Fisher's exact test. A value of $p < 0.05$ was considered to be significant.

Results

Patient characteristics

The 169 patients consisted of 115 men (age range, 24-92 years; median, 72 years) and 54 women (age range, 25-96 years, median, 66.5 years). Most of them had CAP (130 patients), followed by 11 with acute bronchitis, 11 with chronic obstructive pulmonary disease, 9 with bronchiectasis, 4 with secondary infections due to bronchial asthma, 3 with chronic bronchitis, and 1 with acute exacerbation of diffuse panbronchiolitis. *S. pneumoniae* was detected by the sputum culture method in 54 (32.0%) patients. Geckler 4/5 sputa were obtained from 112 patients, Geckler 1-3 from 32 patients, and Geckler 6 from 25 patients.

Comparison of the bacterial counts between the PCR method and the simple quantitative culture method

One-hundred and fifteen of the 169 patients tested culture-negative, and $\pm$ for 2, 1+ for 10, 2+ for 18, and 3+ for 24 patients. The median values of the log-transformed pneumococcal PspA DNA copy number were 3.00 in the *S. pneumoniae*-negative group by the simple quantitative culture method, 6.78 in the $\pm$ /+ group, 7.30 in the 2+ group, and 7.15 in the 3+ group. The PCR-based PspA copy number of the *S. pneumoniae* culture-negative group was significantly lower than that of any of the three groups of $\pm$ or higher counts ($p < 0.01$ for each). Among the *S. pneumoniae* culture-positive groups with different bacterial counts, no significant difference in the PspA DNA copy number was found between any pair.

Comparison of the culture method, various rapid tests, and PCR-based PspA copy number (all patients)

PspA DNA copy numbers were compared between the positive and negative groups of the various rapid tests. In all the rapid tests, the copy number of the positive group was significantly higher than that of the negative group (Gram staining $p < 0.001$, RAPIRUN $p < 0.001$, BinaxNOW $p < 0.01$) (Fig. 1).

Next, the PspA DNA copy numbers were compared between the positive and negative groups of the various rapid tests in *S. pneumoniae* culture-positive patients. For Gram staining and RAPIRUN, the PspA DNA copy number of the positive group tended to be higher than that of the negative group ($p < 0.08$ and $p < 0.07$, respectively). However, no statistical difference was observed with BinaxNOW. Similarly, no significant difference was found in the comparison of PspA DNA copy number between the positive and negative groups of rapid tests in *S. pneumoniae* culture-negative patients (Fig. 1).

The sensitivity, specificity, and concordance rate of the various rapid tests were determined using the status of *S. pneumoniae* detection by the culture method as the standard. Sensitivity was 75.9% (95% confidence interval 62.4-86.5; the same applies below) for Gram staining, 90.7% (79.7-96.9) for RAPIRUN, and 53.7% (39.7-67.3) for BinaxNOW. The sensitivity of BinaxNOW was significantly lower than that of Gram staining and RAPIRUN ($p < 0.03$ and $p < 0.001$, respectively). The sensitivity of RAPIRUN tended to be higher than that of Gram staining ($p < 0.07$). The specificity was 84.3% (76.4-90.4) for Gram staining, 93.0% (86.8-96.9) for RAPIRUN, and 94.8% (89.0-98.0) for BinaxNOW, thus showing that the specificity of Gram staining was significantly lower than that of BinaxNOW ($p < 0.02$) and it tended to be lower than that of RAPIRUN ($p < 0.06$). The concordance rate of RAPIRUN was the highest (92.3%, 87.3-95.8) and significantly higher than that of Gram staining and BinaxNOW (81.7%, 75.0-87.1, for both) ($p < 0.006$) (Fig. 2).

The positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of the various rapid tests were as follows: 69.5, 88.2, 4.9, and 0.29 for Gram staining; 86.0, 95.5, 13.0, and 0.1 for RAPIRUN; and 82.9, 81.3, 10.3, and 0.49 for BinaxNOW, respectively. The positive predictive value of RAPIRUN was significantly higher than that of Gram staining ($p < 0.05$). The negative predictive value of RAPIRUN was significantly higher than that of BinaxNOW ($p < 0.001$) and it tended to be higher than that of Gram staining ($p < 0.06$).

Table 1 shows the sensitivities of the various rapid tests determined for different bacterial counts by the simple quantitative culture method. The sensitivity of RAPIRUN was significantly higher than that of Gram staining when the bacterial count was low ($\pm$ /+) ($p < 0.05$). When the bacterial count was 2+, the sensitivity of Gram staining and RAPIRUN was significantly higher than that of BinaxNOW ($p < 0.05$ and $p < 0.001$, respectively), and when it was 3+, the sensitivity of Gram staining was significantly higher than that of BinaxNOW ($p < 0.03$).

Sixty-one patients had sputa with a PspA DNA copy number of $\geq 10^5$ copies/ μ g of DNA. The positive rates of the various tests were higher in the following order: culture 85.2% (73.9-93.0) > RAPIRUN 82.0% (70.1-90.6) > Gram staining 72.1% (59.2-82.8) >> BinaxNOW 47.5% (34.7-60.7). Moreover, comparison of the detection rates among the various tests revealed the rate of BinaxNOW to be sig-

Figure 1. Results of various rapid tests, culture, and PCR-based PspA copy number. The broken line denotes the lower limit of detection (40 copies/μg of DNA; $\log_{10} 40 = 1.6$). ●: Culture test-positive, ○: culture test-negative. The shaded area indicates patients testing positive in the various rapid testing methods. n is the number of patients, and the value below n is the median (Log) of the PspA DNA copy number.

Figure 2. Sensitivity, specificity, and concordance rate of the various rapid testing methods using the culture method as the standard.

nificantly lower than that for any of the other tests (in the order of comparison with tests with a higher detection rate, $p < 0.001$, $p < 0.001$, and $p < 0.01$) (Fig. 3A). When the same analysis was performed for 30 patients with at least 10^7 copies/μg of DNA, the positive rates of the various tests were in the following order: culture = RAPIRUN 96.7% (82.8-99.9) > Gram staining 80.0% (61.5-92.2) >> BinaxNOW 53.3% (34.4-71.6). Comparison of the detection rates among the various tests revealed that the rate of BinaxNOW was sig-

nificantly lower than that of the culture and RAPIRUN ($p < 0.001$ for both) (Fig. 3B).

Comparison of the sensitivity of the rapid tests by severity and the number of days from onset in the CAP patients

Fig. 4 shows the sensitivities of the various rapid tests in each CAP severity level. Among the 130 CAP patients, the disease was mild in 48, moderate in 65, severe in 15, and

Table 1. Sensitivity of the Various Rapid Tests at Different Bacterial Counts by the Simple Quantitative Culture Method.

Culture	±·1+ n=12	2+ n=18	3+ n=24
Gram staining	25.0% (5.5 — 57.1)	77.8% (52.4 — 93.5)	100.0% (85.8 — 100.0)
RAPIRUN	75.0% (42.9 — 94.5)	100.0% (81.5 — 100.0)	91.7% (73.1 — 98.9)
BinaxNOW	33.3% (10.0 — 65.1)	38.9% (17.3 — 64.2)	75.0% (53.3 — 90.2)

() 95% confidence interval.

In ±·+, RAPIRUN > Gram staining ($p < 0.05$).In 2+, Gram staining > BinaxNOW ($p < 0.05$), RAPIRUN > BinaxNOW ($p < 0.001$).In 3+, Gram staining > BinaxNOW ($p < 0.03$). **Figure 3.** *S. pneumoniae*-positive rate of various detection tests in sputum PspA DNA counts of $\geq 10^5$ copies/ μg of DNA (A) and $\geq 10^7$ copies/ μg of DNA (B). The ratio of copies/ μg of DNA by the PCR measurement system used in this study to the CFU/mL value in the quantitative culture method was almost 1:1.

very severe in 2. Forty-five patients (34.6%) were culture-positive for *S. pneumoniae*, of whom 20 had mild disease, 17 moderate, 6 severe, and 2 very severe. The sensitivities of Gram staining, RAPIRUN, and BinaxNOW were, respectively, 60.0% (36.1-80.8), 90.0% (68.4-98.7), and 45.0% (23.1-68.4) in mild cases; 82.4% (56.6-96.2), 94.1% (71.4-99.8), and 70.6% (44.1-89.6) in moderate cases; and 100.0% (63.1-100.0), 87.5% (47.4-99.6), and 62.5% (24.5-91.4) in severe or very severe cases. The *S. pneumoniae* sensitivity of RAPIRUN in the mild cases was significantly higher ($p < 0.006$) than that of BinaxNOW. *S. pneumoniae* detectability of RAPIRUN was always approximately 90% of that for culture regardless of severity.

The 127 CAP cases with a reported date-of-onset were divided into two groups based on the number of days from onset to specimen collection. The sensitivities of the various rapid tests in each group were determined using the sputum culture results as the standard. Of 71 patients who were tested within 2 days of disease onset, 26 were *S. pneumoniae*-positive in culture. The sensitivities of Gram staining, RAPIRUN and BinaxNOW in these patients were

76.9% (56.4-91.0), 88.5% (69.9-97.5), and 57.7% (37.0-76.6), respectively. Similarly, 56 patients who were tested 3 days after disease onset or later, 19 were *S. pneumoniae*-positive in culture. The sensitivities in these patients were 73.7% (48.8-90.8), 94.7% (74.0-99.8), and 57.9% (33.5-79.7), respectively. In both groups tested within 2 days and after 3 days from onset, the sensitivity of RAPIRUN was significantly higher than that of BinaxNOW ($p < 0.03$ and $p < 0.02$, respectively) (Fig. 5).

Sputum quality and rapid test results

Table 2 shows the sensitivity, specificity, and concordance rate for the various rapid tests stratified by sputum quality. The sensitivity, specificity, and concordance rate of Geckler 4/5 sputum specimens, generally considered to be of good quality, were 82.2%, 85.1%, and 83.9% for Gram staining; 93.3%, 95.5%, and 94.6% for RAPIRUN; and 57.8%, 98.5%, and 82.1% for BinaxNOW, respectively. RAPIRUN showed a significantly higher concordance rate than Gram staining ($p < 0.02$) and a significantly higher sensitivity and concordance rate than BinaxNOW ($p < 0.001$ and $p < 0.006$, respectively). Comparison of the results of Gram staining and BinaxNOW revealed that Gram staining had a significantly higher sensitivity ($p < 0.03$), but BinaxNOW had a significantly higher specificity ($p < 0.01$). The sensitivity of Gram staining was significantly different among the three groups stratified by sputum quality based on the Geckler classification ($p < 0.04$).

Discussion

Respiratory infections due to *S. pneumoniae* tend to rapidly worsen, and early diagnosis and early treatment are critical. In addition, CAP is one of the common diseases seen in primary care. Accordingly, we compared the results of rapid tests for *S. pneumoniae*, Gram staining, RAPIRUN, and BinaxNOW in antibiotic-naïve adults with pneumonia or lower respiratory tract infections using the culture method as a standard procedure.

First, the sputum *S. pneumoniae* counts by PCR and the simple quantitative culture method were compared. Although a large difference was noted in the PCR-based PspA copy number between the culture-negative and -positive groups,

Figure 4. Severity and *S. pneumoniae* detection rate of rapid tests in 45 CAP patients who were positive for *S. pneumoniae* by sputum culture. n is the number of patients.

Figure 5. Comparison of sensitivity of detecting *S. pneumoniae* among rapid testing methods by time point of tests after the onset of pneumonia. n is the number of patients.

no significant difference was observed in the copy number between groups with different bacterial counts as determined by the simple quantitative culture method. These results may be due to the test sample heterogeneity and/or non-viable *S. pneumoniae*, which negatively affect the culture results. Among the culture-positives, the PCR copy numbers for sputum rapid test-positives were higher than those for sputum rapid test-negatives. However, the results of BinaxNOW, which uses urine samples, were completely unrelated to the bacterial count in sputum. These results suggest that BinaxNOW, which uses urine as a sample, does not provide the real-time status of the infection, while sputum produced from the focal infectious site represents the real-time status of the disease.

Next, the performances of the rapid tests were compared using the culture method as the standard. The sensitivity, specificity, and concordance rate of RAPIRUN were all approximately 90%, showing that among the three rapid tests, RAPIRUN provides *S. pneumoniae* detection results closest to those of the culture method. The advantage of Gram staining is that the causative pathogen can be determined within a short time when the technician is experienced, not

only by the morphology and staining, but also by the infiltration of inflammatory cells and phagocytic images. However, in addition to requiring the use of a microscope, the major problem associated with this test is that interpretation of the results varies depending on the experience and skill of the technician. In fact, a meta-analysis has reported a wide range of sensitivity ranging from 15-100% and specificity ranging from 11-100% (7). In our study, all Gram staining specimens were collected at one institution and were interpreted by an expert examiner. Therefore, there was no difference in the interpretation by institutions and individuals. However, the sensitivity and specificity of Gram staining tended to be lower than those of RAPIRUN, and the concordance rate was significantly lower than that of RAPIRUN ($p < 0.006$). Bacteriology expert examiners are not always available in primary care settings (e.g., with general practitioners, in hospitals without laboratory, and in holiday and/or overnight clinics). RAPIRUN has been suggested to be useful in the rapid detection of *S. pneumoniae* with superior sensitivity and specificity even in such situations.

BinaxNOW is widely used, particularly for the presumptive diagnosis of pneumococcal pneumonia. According to a meta-analysis report of CAP in adults, the sensitivity and specificity based on the test results of culture or sputum smears were 75% and 95%, respectively (21). However, the following results were reported for BinaxNOW. The sensitivity for sputum culture tests in pneumonia patients was 23.5% (4/17 patients) (11); of 171 CAP patients testing negative with BinaxNOW, 28 (16.4%) tested *S. pneumoniae*-positive by sputum culture and/or Gram staining (9); and of 12 patients testing *S. pneumoniae*-positive by the culture method and having a gram-positive phagocytic image, 4 tested negative by BinaxNOW (10). These discrepancies are major problems for the detection of *S. pneumoniae*, a pathogen that rapidly worsens the condition of the illness without the initiation of suitable antibacterial therapy. In the present study of 169 antibiotic-naïve patients, only 35 patients tested positive by BinaxNOW, whereas 57 tested positive by

Table 2. Sensitivity, Specificity, and Concordance Rate of the Various Rapid Tests Stratified by Sputum Quality Based on the Geckler Classification.

Geckler Classification		Gram staining	RAPIRUN	BinaxNOW
1-3	sensitivity	50.0% (3 / 6)	66.7% (4 / 6)	33.3% (2 / 6)
	specificity	88.5% (23 / 26)	96.2% (25 / 26)	88.5% (23 / 26)
	concordance rate	81.3% (26 / 32)	90.6% (29 / 32)	78.1% (25 / 32)
4/5	sensitivity	82.2% (37 / 45)	93.3% (42 / 45)	57.8% (26 / 45)
	specificity	85.1% (57 / 67)	95.5% (64 / 67)	98.5% (66 / 67)
	concordance rate	83.9% (94 / 112)	94.6% (106 / 112)	82.1% (92 / 112)
6	sensitivity	33.3% (1 / 3)	100.0% (3 / 3)	33.3% (1 / 3)
	specificity	77.3% (17 / 22)	81.8% (18 / 22)	90.9% (20 / 22)
	concordance rate	72.0% (18 / 25)	84.0% (21 / 25)	84.0% (21 / 25)
Total	sensitivity	75.9% (41 / 54)	90.7% (49 / 54)	53.7% (29 / 54)
	specificity	84.3% (97 / 115)	93.0% (107 / 115)	94.8% (109 / 115)
	concordance rate	81.7% (138 / 169)	92.3% (156 / 169)	81.7% (138 / 169)

Numbers in parentheses indicate the number of patients.

In Geckler 4/5 sputum: sensitivity, RAPIRUN > BinaxNOW ($p < 0.001$); Gram staining > BinaxNOW ($p < 0.02$); specificity, RAPIRUN > Gram staining ($p < 0.08$); BinaxNOW > Gram staining ($p < 0.01$); concordance rate, RAPIRUN > Gram staining ($p < 0.02$); RAPIRUN > BinaxNOW ($p < 0.006$).

Sensitivity of Gram staining was significantly different among the three categories stratified by sputum quality based on the Geckler classification ($p < 0.04$).

RAPIRUN. Sensitivity of sputum culture as the standard was 75.9% for Gram staining, 90.7% for RAPIRUN, and 53.7% for BinaxNOW, showing that the sensitivity of BinaxNOW was significantly lower than that of the other two methods. The low sensitivity of BinaxNOW was also supported by using PCR analysis as a reference. Among the patients with *S. pneumoniae*-positive sputum equal to or greater than 10^5 and 10^7 CFU/mL, considered as the significant pathogen in previous reports (16-19), the positive rates of BinaxNOW were significantly lower than those of RAPIRUN at both bacterial counts ($p < 0.001$).

BinaxNOW detects urinary *S. pneumoniae* antigens that are supposedly derived from the focus of respiratory infection and subsequently enter urine through the blood stream. In this context, we anticipated that the sensitivity of BinaxNOW is lower than that of RAPIRUN, particularly in the early stage of the infection, as RAPIRUN uses sputum produced from the focal inflammatory site. We accordingly stratified the patients into two categories by the number of days from disease onset to sample collection and compared the detection sensitivity of the rapid tests in each category. The sensitivity of BinaxNOW was significantly lower than that of RAPIRUN regardless of the number of days from onset ($p < 0.03$). Based on the results of our detailed analyses, differences between various reports in the positive rates of BinaxNOW may be attributed to differences in the disease severity and the number of days from disease onset.

The general rule in the detection of causative pathogens in pneumonia and respiratory infections is to use good quality sputum that is not contaminated with saliva. In the present study, the sensitivity of RAPIRUN was significantly higher than that of BinaxNOW particularly when good quality Geckler 4/5 sputum was used ($p < 0.001$). The sensitivity and specificity of RAPIRUN tended to be higher than those of Gram staining, and the concordance rate of RAPIRUN was significantly higher than that of Gram staining ($p < 0.02$).

In addition, the sensitivity, specificity, and concordance rate of RAPIRUN were not influenced by the quality of sputum.

These results indicate that RAPIRUN has a higher sensitivity than BinaxNOW as a rapid test for *S. pneumoniae* in patients with pneumonia or lower respiratory tract infection, and gives test results closer to the culture results than Gram staining and BinaxNOW. Unlike Gram staining, RAPIRUN requires no special skill or need to use a microscope and is a simple procedure that can be performed by anyone. Therefore, RAPIRUN appears to be a useful test in primary care.

The limitations associated with this study are as follows; First, the evaluation was mainly of sputum; therefore, the study subjects were limited to patients from whom sputum could be collected. Second, previous histories of pneumococcal pneumonia were not examined. In a follow-up of pneumonia patients testing positive by BinaxNOW, 78% (14/18 patients) have been reported to be positive after 1 month, 50% (5/10 patients) after 2 months, and 60% (3/5 patients) after 3 months, and 2 patients remained positive for more than 6 months (22). We accordingly insist that it is inappropriate to diagnose pneumococcal pneumonia based solely on the positive results of BinaxNOW. The persistent positivity of BinaxNOW due to a previous episode of pneumococcal pneumonia may have increased the percentage of its sensitivity in the present episode. However, the sensitivity and PCR-positive rates of BinaxNOW were lower than those of RAPIRUN. Therefore, BinaxNOW did not increase the diagnostic yield over RAPIRUN in this study.

In conclusion, RAPIRUN was found to be a rapid and reliable diagnostic test with good sensitivity, specificity, and likelihood ratio to detect *S. pneumoniae* in sputum.

We recommend that RAPIRUN, rather than BinaxNOW, should be used as a detection tool in adult pre-treatment patients with suspected pneumonia and expectorating sputum, especially in primary care settings.

Author's disclosure of potential Conflicts of Interest (COI).

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