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Evaluation of a rapid immunochromatographic ODK0501 assay for detecting *Streptococcus pneumoniae* antigen in sputum from patients with lower respiratory tract infection

Running title: Detecting *S. pneumoniae* antigen in sputum

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ABSTRACT

A novel, rapid and noninvasive test (ODK0501) to detect *Streptococcus pneumoniae* antigen was evaluated in a Japanese multicenter study. ODK0501 uses polyclonal antibodies to detect C-polysaccharide of *S. pneumoniae* from sputum by an immunochromatographic assay. The utility of ODK0501 was evaluated in 161 adult patients with lower respiratory tract infection between March 2006 and March 2007. Bacterial culture and identification, real-time PCR, and ODK0501 assays were performed on sputum samples, and Binax NOW *Streptococcus pneumoniae* antigen test was performed in urine samples obtained from the same patients. The performance of all tests was compared based on the results of bacterial culture and identification. The sensitivity and specificity of ODK0501 were 89.1% (49/55) and 95.3% (101/106), respectively. We then compared the Binax NOW *Streptococcus pneumoniae* antigen test with ODK0501 in samples from 142 patients. The sensitivity of ODK0501 and the Binax NOW *S. pneumoniae* antigen test was 90.0% (45/50) and 62.0% (31/50), respectively (P = 0.002). The relative quantity of *S. pneumoniae* in expectorated sputum was calculated using real-time PCR and indicated that the possibility of false-positive results of ODK0501 due to indigenous *S. pneumoniae* was low. The positive and negative concordance rates of ODK0501 and Binax NOW were 96.8% (30/31) and

21.1% (4/19), respectively. Binax NOW was less capable of detecting *S. pneumoniae* antigen among patients with underlying COPD. In conclusion, ODK0501 is non-invasive, rapid and accurate tool for diagnosing respiratory infection caused by *S. pneumoniae*.

INTRODUCTION

Streptococcus pneumoniae is a frequent cause of community-acquired bacterial pneumonia and lower respiratory tract infection (10, 11, 19, 21), and a major cause of significant morbidity and mortality in Japan and the rest of the world (5).

Since pneumococcal infections are common and can be severe, appropriate initial selection of antimicrobial agents is crucial to an optimal outcome. Rapid and precise identification of the causative agents of infectious diseases is critical but challenging. Pathogen-oriented, prompt selection and application of antimicrobial agents improves prognosis, reduces medical costs and prevents the development of drug-resistant bacteria due to their inappropriate use. The gold standard, bacterial culture to identify causative microorganisms requires several days to yield result, and is thus unhelpful to the initial selection of appropriate antibacterial drugs. Although Gleckman et al. reported that classic Gram staining of sputum, which is simple and inexpensive, is useful in the diagnosis of bacterial infections (7), Reed and others found that it is ineffective for rapid diagnoses (6, 18). In addition, staining results rely on several factors such as the quality of the sputum samples and the skill of laboratory personnel in processing samples (7). Thus, Gram staining is not recommended for all patients with community-acquired pneumonia according to the most recent “Infectious Diseases

Society of America/American Thoracic Society (IDSA/ATS) Consensus Guidelines on the Management of Community-Acquired Pneumonia in Adults" (12).

Urinary antigen detection is an alternative rapid diagnostic technique for detecting antigens of *S. pneumoniae* and *Legionella pneumophila* as respiratory pathogens. The Binax NOW *Streptococcus pneumoniae* urinary antigen test (Binax NOW; Binax, Inc., Portland, ME) detects cell wall antigens secreted in urine using an immunochromatographic method to separate capsular polysaccharides (C-ps) of *S. pneumoniae*. The test is noninvasive, rapid (the entire assay can be completed in about 15 minutes) and the specificity and sensitivity of detecting pneumococcal infections in adult patients is > 90% and 50% to 80%, respectively, which allows early diagnosis (3, 8, 16). Moreover, even when *S. pneumoniae* cannot be identified by bacterial culture tests after starting antibiotic therapy, the assay can still detect this organism. However, Binax NOW has significant problems including false-positive results due to indigenous *S. pneumoniae* in children (17) and antigens can be detected even one to three months after treatment (13, 14).

The ODK0501 (Otsuka Pharmaceutical Co., Ltd. Tokyo, Japan) is a diagnostic kit that uses polyclonal antibodies to detect anti-pneumococcal C-ps, a cell wall antigen of *S. pneumoniae* (4). This immunochromatographic method is rapid, noninvasive and

analyzes sputum derived directly from a local infection site. Here, we evaluated the ability ODK0501 to detect *S. pneumoniae* among patients with lower respiratory tract infection at several Japanese medical institutions and compared its performance with that of Binax NOW. The goal of this study is to prove the usefulness of ODK0501 and to its performance in clinical settings.

MATERIALS AND METHODS

Study settings

This case control study proceeded between March 2006 and March 2007 at the following 14 medical institutions in Japan: the Nagasaki University School of Medicine, Saitama Cardiovascular and Respiratory Center, the National Hospital Organization Ureshino Medical Center, Isahaya Health Insurance General Hospital, Hokusho Central Hospital, Japanese Red Cross Nagasaki Genbaku Isahaya Hospital, Kurashiki Central Hospital, Sasebo City General Hospital, Toranomon Hospital, Nakahama Clinic, Ohmichi Internal and Respiratory Medicine, Kitakyushu Municipal Yahata Hospital, University of Tokushima School of Medicine and Shinrakuen Hospital. This study was approved by the Institutional Review Board of each medical institute and the informed consent was obtained from each subject.

All adult inpatients or outpatients (age, ≥ 15 years) with signs of lower respiratory infections including pneumonia and who had undergone sputum exploration were eligible for inclusion in this study. Prior antibiotics use was not a criterion for exclusion. Patients with a provisional diagnosis of lower respiratory infections were assessed by each study investigator to confirm the diagnosis. Clinical information such as existing underlying diseases, prior antibiotics use, number of days from onset until the day when

assays were performed, white blood cell (WBC) counts and C-reactive protein (CRP) levels were recorded when the patients registered and retrospectively analyzed.

Sample collection and microbiological investigations

Single expectorated sputum and urine samples were collected from patients who had provided written, informed consent to participate in this study either at a hospital visit or at the time of a presumptive diagnosis of lower respiratory infection. All expectorated sputum samples were conventionally Gram stained and transferred to the microbiology laboratory at Hokusho Central Hospital for expert evaluation. Sputum samples were also cultured at 37°C for 24 hours on blood and chocolate agar at each institution. Presumptive colonies of bacteria were picked up and identified by biochemical testing. Some portions of the expectorated sputum samples were placed in different containers at the time of sampling, and stored at -20°C until assay with real-time PCR to detect and quantify *S. pneumoniae*.

Samples were immediately evaluated using ODK0501 and Binax NOW, otherwise, stored at 4°C until next available day to perform these tests (all assays were performed within 48 hours after collection of samples).

Soluble *Legionella pneumophila* serogroup 1 antigen in urine was investigated using

another immunochromatographic assay and antibodies against *Mycoplasma pneumoniae*, *Chlamydophila psittaci* and *C. pneumoniae* were examined using complement fixation tests.

Measurement and interpretation of results obtained with ODK0501 and Binax NOW

Each of the participating institutions followed the procedures indicated below.

Sputum samples collected on swabs from containers were shaken in tubes containing sample extract and left for 5 minutes. Swab tips were removed from the tubes while squeezing, a filter was placed on the tube, and the extract was dropped onto the ODK0501 instrument. Approximately 20 minutes later, a positive test result was indicated by the presence of one red test line and one control lines (two lines), whereas a negative result was indicated by the appearance of only the control line.

Results obtained using Binax NOW were measured and interpreted at the each of institutions in accordance with the instructions provided by the manufacturer (3).

Criteria for etiological diagnosis

We evaluated only qualified samples defined according to standard criteria (presence

of > 25 WBC and < 25 squamous cells per low-power magnification field [× 100]). The following criteria were used to classify a case of lower respiratory infections as being of known etiology: (ⅰ) *M. pneumoniae C. psittaci* and *C. pneumoniae*, a ≥ 4-fold increase in antibody, as determined by the complement fixation test; (ⅱ) Legionella antigen detected in urine, (ⅲ) for *S. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus* and other bacteria including gram-negative enterobacteria, isolation of the pathogen of the predominant organism from a qualified sputum sample. Patients who did not fulfill the etiologic diagnostic criteria described above were considered to have lower respiratory infection of unknown etiology.

Real-time PCR for pneumococcal surface protein A (Psp A) gene quantitation in expectorated sputum samples

We extracted DNA from expectorated sputum samples using the QIAamp® DNA Mini Kit (Qiagen, Valencia, CA) and the relative amount of *S. pneumoniae* was quantified by real-time PCR using a primer and TaqMan probe established for the region of the Psp A gene (2, 22) of *S. pneumoniae* (Table 1). Real-time PCR proceeded on an ABI7700 or ABI7900 (Applied Biosystems, Foster City, CA) at Kitazato-Otsuka Biomedical Assay Laboratory, Kanagawa, Japan and sequences were analyzed using Sequence Detector V.

1.7a, V.2.2.2 SDS (Applied Biosystems, Foster City, CA). RNase free water and the extracted DNA from *S. pneumoniae* ATCC6303 were used for negative and positive control, respectively. After initial denaturation at 95°C for 15 minutes, PCR products were amplified by 50 cycles of 15 seconds at 94°C and 1 minute at 60°C. Copy numbers of the Psp A gene were calculated and compared among ODK0501- and culture-positive and -negative sputum samples.

Statistical analysis

The sensitivity and specificity based on the results of the sputum cultures of ODK0501 and Binax NOW was compared using Fisher's exact probability test (two-tailed, level of significant difference $P < 0.05$). Characteristics of patients with pneumococcal infection diagnosed by both culture and ODK0501 as positive were also compared with Binax NOW-positive or -negative arms. We compared the proportions of qualitative variables using Fisher's exact test, and applied the Mann-Whitney-U test for quantitative variables. Relationships between the results obtained from real-time PCR for relative amounts of *S. pneumoniae* among the ODK0501 tested population were also assessed using the Mann-Whitney-U test.

RESULTS

Composition of patients and etiological agents identified from patients with lower respiratory tract infection

Of the 246 patients exhibiting signs and symptoms compatible with lower respiratory infection, 85 were excluded due to poor sputum quality (presence of either or both of < 25 WBC and > 25 squamous cells per low-power magnification field [× 100]).

We thus investigated samples from 161 inpatients and outpatients (109 males: age range, 22 to 92 y; median age, 71.0 y, 52 females: age range, 15 to 96 y; median age, 65.5 y) with lower respiratory tract infection. Table 2 shows the clinical status of the patients. Most had pneumonia (79.5%), followed by acute exacerbation of chronic obstructive pulmonary disease (AECOPD) (7.5%), acute bronchitis (6.2%), bronchiectasis (5.0%), chronic bronchitis (1.2%) and only one had a lung abscess. We examined samples from 161 and 142 patients using ODK0501 and Binax NOW, respectively. Table 2 also shows the etiological agents. According to conventional microbiological criteria, etiological agents were identified in 106 patients (65.8%). The following were detected and determined as causative agents: *S. pneumoniae* in 55 (34.2%), *Haemophilus influenzae* in 25 (15.5%), *M. pneumoniae* in 5 (3.1%), *Staphylococcus aureus* in 8 (5.0%), *Pseudomonas aeruginosa* in 7 (4.3%), *Moraxella catarrhalis* in 5 (3.1%), and

Klebsiella pneumoniae in 2 (1.2%). Four patients with mixed *S. pneumoniae* and *H. influenzae* infection were in the pneumonia and AECOPD groups. Two other patients in the pneumonia group had mixed infection due to *S. pneumoniae* and *C. pneumonia*e, and to *M. pneumoniae* and *P. aeruginosa*. The microbial etiology remained unknown after conventional microbiological culture and antibody test in 55 (34.2%) of the 161 patients.

Overall sensitivity and specificity of ODK0501

The ODK0501 results were positive in 54 of 161 patients (33.5%). Table 2 shows the distribution of ODK0501 positivity in each patient group. ODK0501 was positive in 41 (87.2%) of 47 patients with pneumococcal pneumonia including four patients with mixed infections and in all patients with AECOPD (n = 4), acute bronchitis (n = 1), bronchiectasis (n = 1) and chronic bronchitis (n = 1) caused by *S. pneumoniae*. The test sensitivity was 89.1% (49/55), the specificity was 95.3% (101/106) and the positive and negative predictive values were 90.7% and 94.4%, respectively, based on the results of the sputum cultures.

Comparison of sensitivity and specificity between ODK0501 and Binax NOW

We compared the sensitivity and specificity between ODK0501 and Binax NOW against sputum culture results from 142 of the 161 patients (Binax NOW was not performed in 19 of 161 patients).

The ODK0501 test sensitivity was 90.0% (45/50), the specificity was 95.7% (88/92) and the positive and negative predictive values were 91.8% and 94.6%, respectively**,** based on the sputum cultures. The Binax NOW test sensitivity was 62.0% (31/50), the specificity was 96.7% (89/92) and the positive and negative predictive values were 91.2% and 82.4%, respectively**,** based on the sputum cultures. Specificity did not significantly differ between the two tests, but the sensitivity of ODK0501 for detecting *S. pneumoniae* was significantly higher (P = 0.002).

Quantification of Psp A gene in sputum by real-time PCR

We applied real-time PCR to detect and quantify the relative amount of *S. pneumoniae* in expectorated sputum samples from 161 patients with lower respiratory tract infection. Figure 1 shows that Psp A gene copy numbers were higher among the ODK0501-positive and culture-positive population. The population that was both ODK0501- and culture-negative was distributed in the lower copy number of the Psp A gene in sputum. The copy numbers of the Psp A gene significantly differed among

ODK0501-positive and -negative populations ($P < 0.001$).

False-positive and false-negative ODK0501 results among patients with lower respiratory infection

Table 3 summarizes the clinical and laboratory data of the five *S. pneumoniae* culture-negative patients with lower respiratory infection and apparent false-positive ODK0501 results. Binax NOW was positive and the Psp A gene copy number was $> 6 \times 10^6$ in same two patients (Patients 2 and 4).

Table 4 summarizes the clinical and laboratory data from 6 patients with lower respiratory infection who generated false-negative results on ODK0501 but were positive in *S. pneumoniae* cultures. All of these patients had pneumonia and Binax NOW was positive in only one of them (Patient 3). All of these patients had $> 1 \times 10^5$ copies of the Psp A gene.

Comparison between ODK0501 and Binax NOW among patients with pneumococcal infections

We compared the performance of ODK0501 and Binax NOW among 50 patients with pneumococcal infection. Five of 55 pneumococcal patients were excluded due to the

absence of Binax NOW data. The positive and negative concordance rates of ODK0501 and Binax NOW were 96.8% (30/31) and 21.1% (4/19), respectively.

Fifteen patients were ODK0501 positive and Binax NOW negative. Table 5 summarizes their clinical and laboratory findings of ODK0501-positive population among pneumococcal infection proved by culture test. Age, gender, final diagnosis, underlying disease, prior antibiotics use, WBC count, CRP level, copy numbers of Psp A gene and days from onset until assay did not significantly differ between Binax NOW-positive and -negative populations. However, the ODK0501-positive and Binax NOW-negative population contained statistically more patients with COPD than the population that was positive in both tests (P=0.016). Only Patient 3 was ODK0501 negative and Binax NOW positive (Table 4).

DISCUSSION

Although early diagnosis and antimicrobial drug administration comprise the basic principles of treating pneumococcal as well as other infections, the causative agents may not be correctly identified in many patients at the start of therapy.

Binax NOW is commercially available and widely used in clinical settings, but it possess some disadvantages including false-positive results due to indigenous *S. pneumoniae* (17), lasting positivity even 1 - 3 months after treatment (13, 14) and no indication for anuric patients. Furthermore, the test has a variable and relatively low sensitivity of 50 to 80% (3, 8, 16). The novel ODK0501 tests sputum samples derived directly from inflammatory sites using polyclonal antibodies to detect anti-pneumococcal C-ps (20), a common cell wall antigen of *S. pneumoniae* (4). Thus, all types of *S. pneumoniae* can be detected regardless of capsule serotype.

The present clinical study evaluated the applicability of ODK0501 and compared with Binax NOW in adult patients (age, ≥ 15 years). The most frequent isolate in the present study was *S. pneumoniae* (34.7%) followed by *H. influenza* among 161 patients with lower respiratory tract infection. These findings indicated that the frequency of major isolated bacteria from patients with lower respiratory tract infection was similar to those of other studies, and thus our patient population was valid for analysis.

ODK0501 yielded favorable results including sensitivity and specificity of 89.1% and 95.3%, respectively, based on sputum culture results from 161 patients. Even when analyzed a total of 120 cases with more superior quality of expectorated sputum (presence of both of > 25 WBC and < 10 squamous cells per low-power magnification field [× 100]) (data not shown), the result showed that higher sensitivity (92.9 %) and almost equal specificity (93.6 %). The sensitivity (87.2%) and specificity (95.1%) was also favorable when evaluated among 128 pneumonia cases only.

The sensitivity of ODK0501 was significantly higher (P = 0.002) and the specificity was >95% when compared with Binax NOW. The negative predictive value of Binax NOW was also lower than that of ODK0501. These data indicated ODK0501 is superior to Binax NOW. However, because *S. pneumoniae* can be indigenous to the nasal cavity, nasopharyngeal and intraoral regions of some of the elderly, infants and children (1, 9) sputum or nasopharyngeal samples obtained from such individuals might be *S. pneumoniae*-positive with ODK0501. The question will be raised that the higher sensitivity of ODK0501 suggests that indigenous, colonizing *S. pneumoniae* in the respiratory tract detected by this method will be considered as the cause of infection and lead to over diagnosis. To answer this question, we measured amounts of *S. pneumoniae* in expectorated sputum. Psp A has being originally considered as a potential antigen and

virulence factor of *S. pneumoniae* (15) and was recently applied for epidemiological analysis of *S. pneumoniae* infection (2). Since single copy of Psp A gene exist per *S. pneumoniae* cell and the copy number in sputum relatively correlated to the numbers of *S. pneumoniae* cells (data not shown), we evaluated Psp A gene copy numbers in sputum using real-time PCR. The PCR data were then substituted as relative amounts of *S. pneumoniae* in sputum samples. However, because the amounts of sputum samples varied, values were corrected using human DNA concentrations in the sputum, and copies/μg DNA (calculated based on human DNA concentrations in sputum) was used as the units of measurement. The result for quantitative cultures using an adjusted bacterial suspension as the measurement sample of 9.45×10^5 CFU/mL was nearly equivalent to 4.3×10^5 copies/μg DNA, indicating a nearly 1:1 relationship (data not shown). Most ODK0501-negative patients also had negative culture results as well as less *S. pneumoniae*. Hence, ODK0501-positive patients also had positive culture results and more *S. pneumoniae* according to real-time PCR. These data suggested that ODK0501 does not detect low amount of *S. pneumoniae,* which translates as a low false-positive rate in the presence of indigenous *S. pneumoniae*. Real-time PCR data also supported the finding that the analytical sensitivity of ODK0501 that was approximately 10^4 CFU/ml in the development phase (4) was appropriate to detect *S.*

pneumoniae as the cause of lower respiratory infection.

Analysis of the clinical characteristics of five ODK0501 false-positive patients revealed no specific factors that could account for this result. However, Binax NOW was positive in two patients in whom we detected 6×10^6 copies of the Psp A gene, suggesting that culture did not detect *S. pneumoniae* for reasons that remain unknown. The severity of pneumonia among the 5 patients scored as defined in the "The Japanese Respiratory Society Guidelines for the Management of Community–Acquired Pneumonia in Adults" did not differ (data not shown).

Six patients were ODK0501 false-negative and their clinical backgrounds also did not offer any apparent explanations. Only one patient was Binax NOW-positive, but 4 were Binax NOW negative, indicating that Binax NOW does not offer any advantages as a rapid diagnostic tool for pneumococcal infection.

Among the 50 patients with *S. pneumoniae* infection diagnosed by culture, 15 (30.0%) were Binax NOW-negative and ODK0501-positive, indicating that ODK0501 possess more power to detect *S. pneumoniae* antigen than Binax NOW. We investigated which clinical factors were related to the higher sensitivity of ODK0501 in diagnosing pneumococcal infections. We compared the clinical backgrounds of ODK0501- positive and the Binax NOW-positive arm, and the ODK0501-positive and Binax

NOW-negative arm. We found that only underlying COPD statistically differed among all tested factors, though the number of COPD cases was only thirteen. In these COPD patients, age, prior antibiotics use, WBC count, CRP level, copy numbers of Psp A gene and days from onset until assay were compared and no significant differences between Binax-positive and –negative populations were found (data not shown). Although whether the detection rate of Binax NOW decreases in COPD patients remains unknown, ODK0501 was more sensitive in the present study.

The relationships between the severity of pneumonia and the sensitivity of ODK0501 and Binax NOW are also important. We compared the performance of ODK0501 and Binax NOW with the severity of pneumonia scored according to the definitions in the "The Japanese Respiratory Society Guidelines for the Management of Community-Acquired Pneumonia in Adults" among patients with community-acquired pneumonia and found no significant differences between the detection rates of pneumococcal pneumonia at any level of severity between the two assays (data not shown).

In conclusion, since ODK0501 evaluates sputum sampled from local sites of infection, the results reflect the real-time status of infection due to *S. pneumoniae*. Our study indicated ODK0501 was statistically more sensitive than the Binax NOW *Streptococcus*

pneumoniae urinary antigen test, suggesting that ODK0501will significantly contribute to the early, rapid and non-invasive diagnosis of pneumococcal infection in adult.

CONFLICT OF INTERESTS

Conflicts of interest of all authors are none.

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TABLE 1. Primers used for real-time PCR for quantification of *Streptococcus pneumoniae.*

Forward primer	5’-CAAGTCTAGCCAGCGTCGCTAT-3’
Reverse primer	5’-GGGAGATTCTTCTGCTCTTACAAAAG-3’
	5’-GGGAGATTCTTCTGCTCTTACCAAAG-3’
	5’-GGGAGATTCTTCTGCTCTTACAACAG-3’
TaqMan probes	5’-(FAM)-CTGAGACGCAACAAAACCAGCCCC-(TAMRA)-3’
	5’-(FAM)-CTGAGACGTAACAAAACCAGCCCC-(TAMRA)-3’
	5’-(FAM)-CGAAGACGCAACAAAACCAGCCCC-(TAMRA)-3’

TABLE 2. The patient condition group and ability of ODK0501 to detect *Streptococcus pneumoniae*.

Patient condition group	Number of patients (%)	Ratio (%) of ODK0501+ patients/total patients	
Pneumonia	128(79.5)		
Streptococcus pneumoniae		37/43	(86.0)
Haemophilus influenzae		1/16	(6.3)
S. pneumoniae and *H. influenzae*		3/3	(100.0)
Staphylococcus aureus		0/7	
Moraxella catarrhalis		0/3	
Pseudomonas aeruginosa		0/3	
Klebsiella pneumoniae		0/2	
Streptococcus milleri group		0/1	
Mycoplasma pneumoniae		0/4	
Aspergillus spp.		0/1	
Legionella pneumophila		0/1	
Chlamydophila psittaci		0/1	
S. pneumoniae and *C. pneumoniae*		1/1	(100.0)
M. pneumoniae and *P. aeruginosa*		0/1	
Pathogen not identified		3/41	(7.3)
Acute exacerbation of chronic obstructive pulmonary disease	12 (7.5)		

S. pneumoniae		4/4	(100.0)
H. influnezae		0/2	
S. pneumoniae and *H. influenzae*		1/1	(100.0)
M. catarrhalis		0/2	
Pathogen not identified		0/3	
Acute bronchitis	10 (6.2)		
H. influnezae		0/2	
S. pneumoniae		1/1	(100.0)
S. aureus		0/1	
Pathogen not identified		0/6	
Bronchiectasis	8 (5.0)		
P. aeruginosa		0/3	
S. pneumoniae		1/1	(100.0)
Pathogen not identified		0/4	
Chronic bronchitis	2 (1.2)		
S. pneumoniae		1/1	(100.0)
Pathogen not identified		1/1	(100.0)
Lung abscess	1 (0.6)		
H. influnezae		0/1	
Total	161	54/161	

TABLE 3. Summary of clinical and laboratory data for ODK0501 false-positive patients.

	Final diagnosis	Gender, age	Underlying disease	Prior antibiotic therapy	Sputum findings	Binax NOW *S. pneumoniae* antigen test	*S. pneumoniae* Real time PCR (copies/μg DNA)	Time from symptom onset until assay performance (days)
1	Pneumonia	M, 43	Bronchial asthma	None	ND[d]	−	2×10^6	4
2	Pneumonia	M, 79	COPD	SBT/ABPC[a]; CAM[b]	*Aspergillus* spp.	+	6×10^6	1
3	Pneumonia	M, 62	Bronchiectasis	None	*Haemophilus influnezae*	−	7×10^2	2
4	Pneumonia	F, 90	None	CTM-HE[c]	ND	+	3×10^7	6
5	Chronic bronchitis	F, 50	None	None	Mixed oral flora	−	1×10^4	3

[a] SBT/ABPC, sulbactam/ampicillin; [b] CAM, clarithromycin; [c] CTM-HE, cefotiam hexetil; [d] ND, not detectable.

TABLE 4. Summary of clinical and laboratory data for ODK0501 false-negative patients.

	Final diagnosis	Gender, age	Underlying disease	Prior antibiotic therapy	Gram stain (*S. pneumoniae*[a])	Binax NOW *S. pneumoniae* antigen test	*S. pneumoniae* Real time PCR (copies/μg DNA)	Time from symptom onset until assay performance (days)
1	Pneumonia	M, 28	None	None	+	−	6×10^6	1
2	Pneumonia	M, 65	COPD	None	−	−	1×10^6	3
3	Pneumonia	M, 33	None	CAM[b];CAZ[c]	+	+	9×10^6	5
4	Pneumonia	F, 33	None	None	−	−	5×10^5	2
5	Pneumonia	M, 42	Interstitial pneumonia	SBTPC[d]	+	Not tested	4×10^5	2
6	Pneumonia	M, 64	Collagen disease	CTRX[e]	−	−	1×10^5	6

[a] *Streptococcus pneumoniae;* [b] CAM, clarithromycin; [c] CAZ, ceftazidime; [d] SBTPC, sultamicillin; [e] CTRX, ceftriaxone.

TABLE 5. Comparison of clinical and laboratory data between ODK0501+ and Binax NOW-positive arm, and ODK0501+ and Binax NOW-negative arm.

Characteristic	ODK0501(+) and Binax NOW (+) (n = 30)	ODK0501(+) and Binax NOW (-) (n = 15)	*P*
Age, median years (range)	67 (22-86)	72 (34-84)	0.718
Male gender	21 (70.0)	14 (93.3)	0.129
Final diagnosis			
Pneumonia	28 (93.3)	12 (80.0)	0.315
Bronchiectasis	1 (3.3)	0 (0.0)	1
Acute exacerbation of COPD	1 (3.3)	1 (6.7)	1
Acute bronchitis	0 (0.0)	1 (6.7)	1
Chronic bronchitis	0 (0.0)	1 (6.7)	1
Underlying disease*	18 (60.0)	10 (66.7)	0.752
COPD	5 (16.7)	8 (53.3)	0.016
Diabetes	3 (10.0)	1 (6.7)	1
Chronic lung disease	0 (0.0)	0 (0.0)	1
Chronic heart disease	1 (3.3)	0 (0.0)	1
Chronic liver disease	1 (3.3)	0 (0.0)	1
Chronic renal insufficiency	0 (0.0)	0 (0.0)	1
Cancer	2 (6.7)	1 (6.7)	1
Dementia	0 (0.0)	0 (0.0)	1
Prior antibiotic therapy	5 (16.7)	0 (0.0)	0.153
CRP, average (range) (mg/dl)	14 (0.3-31.5)	11 (2.0-24.0)	0.448
WBC, average (range) (/mm^3)	12,224	13,723	0.754

	(2,800-31,700)	(6,800-48,800)	
S. pneumoniae real time PCR (range)	1.6×10^7	1.8×10^7	0.431
(copies/μg DNA)	(2×10^3-9×10^7)	(2×10^5-7×10^7)	
Time from onset of symptoms until assay (days) (range)	4 (0-10)	2 (0-9)	0.420

COPD, Chronic obstructive pulmonary disease; Data are ratios (%) of patients, unless otherwise indicated. *One or more of these conditions.

Figure legend

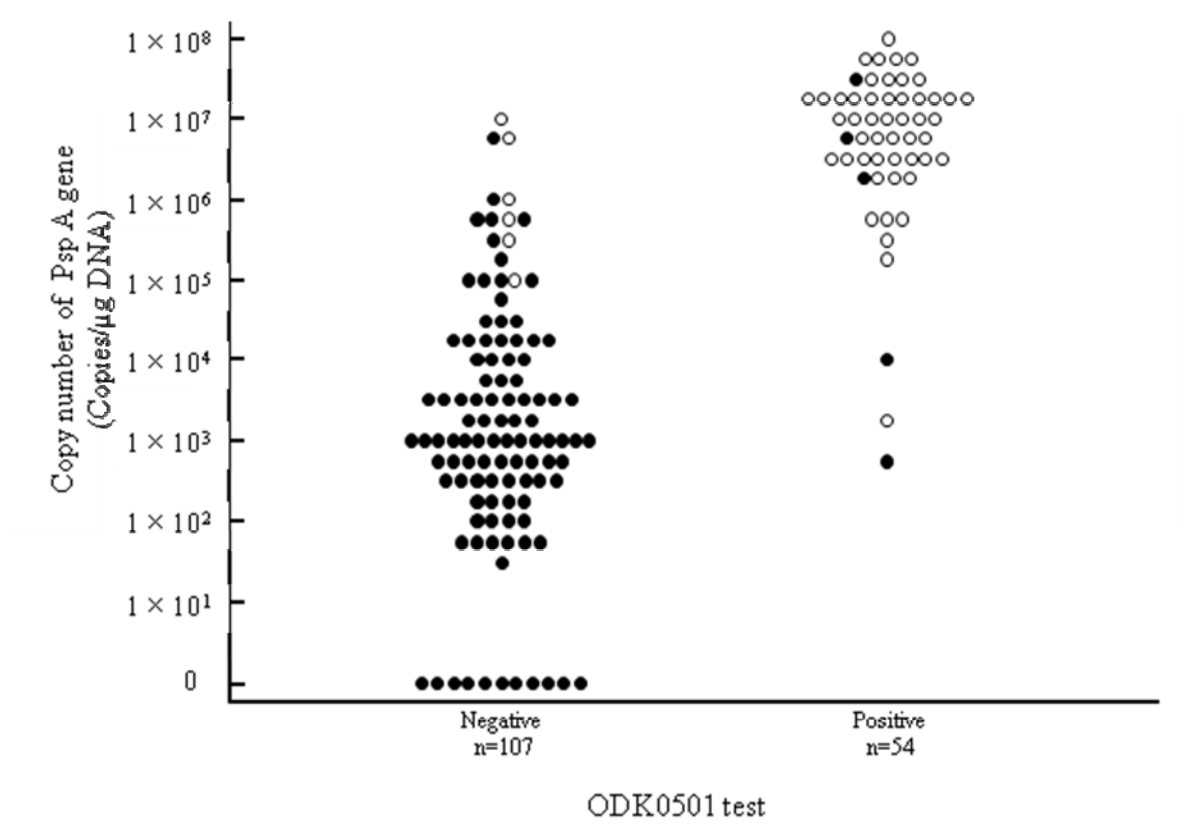

FIG. 1. Distribution of *S. pneumoniae* Psp A gene copy number in sputum compared among ODK0501- and culture positive and -negative populations.

Vertical axis, copy number of Psp A gene calculated from real-time PCR. ●, *S. pneumoniae* culture-negative; ○, *S. pneumoniae* culture-positive. ODK0501-positive and culture-positive populations contain more copies of *S. pneumoniae* Psp A gene. Copy numbers significantly differed between ODK0501-positive and -negative populations ($P < 0.001$).