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Original article

# Rapid diagnostic method for the identification of *Mycoplasma pneumoniae* respiratory tract infection

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ABSTRACT

Rapid diagnostic tests are useful tools in the early diagnosis of respiratory tract infections (RTIs) caused by a specific pathogens. We investigated the sensitivity and specificity of a rapid and simple antigen test for the detection of *Mycoplasma pneumoniae*, Ribotest Mycoplasma® in adolescent and adult patients with RTIs. In addition, we evaluated the accuracy of clinical and laboratory findings for the early presumptive diagnosis of *M. pneumoniae* RTI. We compared 55 cases with laboratory-confirmed *M. pneumoniae* infection using serology, culture, and polymerase chain reaction (PCR) and 346 cases without laboratory-confirmed *M. pneumoniae* infection. Pneumonia cases were excluded in this study. Among patients with *M. pneumoniae* infection, the incidences of cough, sore throat, and sputum production were high, with rates of 98%, 61%, and 67%, respectively, but the specificity was low. The prevalence of nasal symptoms was significantly lower in patients with *M. pneumoniae* infection (9%) than in non-*M. pneumoniae* infection (70%; $p < 0.0001$). When PCR was used as the control test, the sensitivity, specificity, and overall agreement rates with Ribotest® were 71%, 89%, and 87%, respectively. Clinical symptoms and laboratory data were of limited value in making the diagnosis of *M. pneumoniae* RTI in adolescent and adult patients. Our results suggested that Ribotest® may be helpful in distinguishing *M. pneumoniae* RTI patients from those without the disease. Physicians should consider the use of Ribotest® when patients have a persistent cough without nasal symptoms.



## 1. Introduction

*Mycoplasma pneumoniae* is a well-recognized respiratory pathogen principally affecting children and younger adults [1,2]. Pneumonia severity with *M. pneumoniae* is usually mild, but it is well-known that some cases develop into refractory or fulminant pneumonia [1,3]. In addition, macrolide-resistant *M. pneumoniae* with mutations in the 23S rRNA gene have become widespread, especially in Asian countries [4–6]. The clinical and bacteriological efficacy of macrolides for treating cases of macrolide-resistant *M. pneumoniae* was lower than in cases of macrolide-sensitive *M. pneumoniae* [7–12]. This situation demonstrates that the specific diagnosis of *M. pneumoniae* infection is important.

A rapid antigen kit for the detection of *M. pneumoniae* L7/L12 ribosomal protein using an immunochromatographic assay, Ribotest Mycoplasma® (Asahi Kasei Pharma Co., Tokyo, Japan), became available in Japan in 2013 [13–15]. However, available data on the sensitivity and specificity of this kit are limited to children or pneumonia [15,16].

The main purpose of the present study was to identify a means of rapidly distinguishing *M. pneumoniae* respiratory tract infection (RTI) from other causes of RTI in daily clinical practice without waiting for serological, culture or polymerase chain reaction (PCR) results. We evaluated the accuracy of clinical and laboratory findings for the early presumptive diagnosis of *M. pneumoniae* RTI. An additional purpose of this study was to investigate the sensitivity and specificity of Ribotest Mycoplasma® in adolescent and adult patients with *M. pneumoniae* RTI. We compared it with real-time PCR and serology as control tests.

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## 2. Patient and methods

### 2.1. Study populations

Between April 2015 and August 2015, an epidemic of *M. pneumoniae* infection occurred in some areas of Western Japan. This study was conducted a five institutions, Kawasaki Medical School Kawasaki Hospital, Kurashiki Daiichi Hospital, Kurashiki Heisei Hospital, Kurashiki Memorial Hospital, and Kasaoka Daiichi Hospital, between May 2015 and August 2015. We enrolled adolescent (16–19 years-old) and adult patients (≤65 years-old) who had at least two RTI symptoms and did not show any radiographic pulmonary abnormalities by chest X-ray. Clinical symptoms of RTI included nasal symptoms (sneezing, nasal mucus, and nasal congestion), sore throat, cough, sputum production, chest pain, dyspnea, arthralgia, headache, or history of fever. We also enrolled patients who were exposed to RTI symptoms by a family member or work/school colleague if the patients had one of the above RTI symptoms. Blood tests for peripheral white blood cell count, alanine aminotransferase, and aspartate aminotransferase, lactate dehydrogenase and C-reactive protein were performed before appropriate antibiotic therapy. Informed consent was obtained from all patients, and the study protocol was approved by the Ethics Committee of Kawasaki Medical School (approval number 2195).

### 2.2. Microbiological laboratory tests

Microbiological tests, such as Gram stain, cultures, real-time PCR, antigen detection tests, and serological tests, were performed as described previously [17]. Ribotest Mycoplasma® was performed in accordance with the manufacturer's instructions. *M. pneumoniae* infection was diagnosed by a positive culture or PCR result or a fourfold increase in antibody titer, as reported previously [12,17]. Cultivation of *M. pneumoniae* was carried out using pleuropneumonia-like organism broth (Difco, Detroit, MI, USA). DNA then was extracted using a QIAamp DNA Mini Kit (QIAGEN K. K., Tokyo, Japan) in accordance with the manufacturer's instructions. *M. pneumonia* DNA was detected by a real-time PCR targeting a conserved part of the gene encoding P1 adhesin. Antibodies to *M. pneumoniae* were measured using a particle agglutination test (Serodia-Myco II kit, Fujirebio, Tokyo, Japan).

### 2.3. Detection of point mutations for macrolide resistance in domain V of 23S rRNA

A search for mutations at sites 2063, 2064, and 2617 in the *M. pneumoniae* 23S rRNA domain V gene region was performed using a direct sequencing method in isolates or samples with a positive PCR result, as reported previously [8,9]. Specifically, nested PCR was performed using a thermal cycler (PCR Thermal Cycler Dice Gradient, Takara Bio, Inc., Shiga, Japan) with primers (Sigma–Aldrich, Japan), Taq polymerase (Takara Ex *Taq* Version; Takara Bio, Inc.) and extracted DNA. The PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN). The purified products were electrophoresed in a 3% Nusieve 3:1 agarose gel (Lonza) and, after the single band was confirmed, labeled using a BigDye Terminator V3.1 cycle sequencing kit (Applied Biosystems) and applied to an ABI Prism 3130 × 1 Genetic Analyzer (Applied Biosystems) in accordance with the manufacturer's instructions. The presence or absence of gene mutations at each site was determined using a sequence scanner (Applied Biosystems). Patients infected with *M. pneumoniae* showing a point mutation in domain V of the 23S rRNA gene were defined as patients with macrolide-resistant *M. pneumoniae*.

### 2.4. Statistical analysis

Statistical analysis was performed using Stat View version 5.0. (SAS Institute Inc, Cary, NC, USA). The incidence of clinical findings of patients with and without laboratory evidence of *M. pneumoniae* was compared using Fisher's exact test. The mean age of patients and laboratory data were compared using Student's *t* test.

## 3. Results

### 3.1. Patient characteristics

During the study period, 548 patients visited our hospital with complaints of RTI symptoms. Of these patients, physicians could not follow-up or could not carry out all microbiological tests for 147 patients. Finally, 401 patients were assessed using all the microbiological tests described in the Methods section. Of these, 55 cases were diagnosed as laboratory-confirmed *M. pneumoniae* infection: three were culture positive, 46 were PCR positive, and 37 demonstrated positive serological results. Three culture-positive cases were also PCR-positive and serology-positive. Twenty-eight cases were positive in both PCR and serology. When PCR was used as the gold standard, the sensitivity, specificity, and overall agreement with serology were 60.8%, 97.4%, and 93.2%, respectively. Thirty-one cases that were only positive using Ribotest® were excluded from laboratory-confirmed *M. pneumoniae* infection because Ribotest® shows an approximately 10% false-positive rate [15,16].

Twenty-eight of 46 patients with *M. pneumoniae* PCR-positive were found to be infected with macrolide-resistant *M. pneumoniae*. Among these 28 macrolide-resistant patients, 27 had an A-to-G transition at position 2063 in domain V on the 23S rRNA gene (A2063G) and one had an A-to-G transition at position 2064 (A2064G). No mutations at site 2617 in domain V of the 23S rRNA gene were observed.

Table 1 shows the characteristics of the patients with and without laboratory-confirmed *M. pneumoniae* infection. No significant differences in age, gender, or co-morbid illnesses were identified between the two groups, but patients with laboratory-confirmed *M. pneumoniae* infection were more likely to have cough (24 vs 61, $p < 0.0001$) and sore throat (31 vs 119, $p = 0.0025$) and less likely to have nasal symptoms (0 vs 225, $p < 0.0001$) at onset of their RTI (data not shown). The clinical findings observed during RTI, patients with laboratory-confirmed *M. pneumoniae* infection were more likely to have cough, sore throat, and sputum production and less likely to have nasal symptoms. No significant differences in laboratory data were found between the two groups, and mean white blood cell, alanine aminotransferase, aspartate aminotransferase, and lactose dehydrogenase levels were normal in both groups.

### 3.2. Sensitivity and specificity of Ribotest® in patients with RTI

The correlations between Ribotest® and real-time PCR results using 401 RTI cases are presented in Table 2. Ribotest® was positive in 69 cases; 33 cases were PCR positive, and 36 cases were PCR negative. When PCR was used as the control test, the sensitivity, specificity, and overall agreement with Ribotest® were 71.7%, 89.8%, and 87.7%, respectively.

Among the 69 Ribotest®-positive cases, 20 cases were serology positive and 49 cases were serology negative (Table 3). When serology was used as the control test, the sensitivity, specificity, and overall agreement with Ribotest® were 54.0%, 86.5%, and 83.5%, respectively. Two serology-positive cases were observed in 36 PCR-negative and Ribotest®-positive cases.