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Evaluation of a novel urinary antigen test kit for diagnosing *Legionella* pneumonia

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ABSTRACT

Objectives: The aim of this study was to evaluate the diagnostic utility of a novel test kit that could theoretically detect all serogroups of *Legionella pneumophila* for diagnosing *Legionella* pneumonia, in comparison with existing kits.
Methods: This study was conducted in 16 hospitals in Japan from April 2016 to December 2018. Three urinary antigen test kits were used: the novel kit (LAC-116), BinaxNOW *Legionella* (Binax), and Q-line Kyokutou *Legionella* (Q-line). In addition, sputum culture and nucleic acid detection tests and serum antibody tests were performed where possible. The diagnostic accuracy and correlations of the novel kit with the two existing kits were analyzed.
Results: In total, 56 patients were diagnosed with *Legionella* pneumonia. The sensitivities of LAC-116, Binax, and Q-line were 79%, 84%, and 71%, respectively. The overall match rate between LAC-116 and Binax was 96.8% and between LAC-116 and Q-line was 96.4%. One patient had *L. pneumophila* serogroup 2,

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and only LAC-116 showed a positive result, whereas Binax and Q-line did not.
Conclusions: The novel *Legionella* urinary antigen test kit was useful for diagnosing *Legionella* pneumonia. In addition, it could detect *Legionella* pneumonia caused by non-*L. pneumophila* serogroup 1.

Introduction

Legionella pneumonia is caused by *Legionella* species, and there are 58 *Legionella* species and more than 70 serogroups (SGs) (Burillo et al., 2017). Of the *Legionella* species, *L. pneumophila* is the most common causative pathogen of disease in humans and includes 15 SGs (Phin et al., 2014). *Legionella* pneumonia has been reported to account for 1–10% of cases of community-acquired pneumonia (CAP) (Ishida et al., 1998; Lim et al., 2001; Saito et al., 2006; von Baum et al., 2008; Cillóniz et al., 2011), but this percentage is up to 10–15% in severe CAP cases (Ishiguro et al., 2013; Arancibia et al., 2014; Ishida et al., 2014). Therefore, *Legionella* pneumonia is an important cause of severe CAP. Furthermore, the mortality rate is relatively high in such cases, unless the patient receives early and appropriate antibiotic treatment (Heath et al., 1996).

Various examinations are performed for the diagnosis of *Legionella* pneumonia, including urinary antigen tests, respiratory specimen cultures, nucleic acid detection tests, and serum antibody tests. Although culture of *Legionella* species from a respiratory specimen is the diagnostic gold standard (Fields et al., 2002), this requires a specific culture medium, such as buffered charcoal yeast extract-α (BCYE-α) or Wadowsky–Yee–Okuda-α (WYO-α), and identification takes about 3–5 days (Cunha et al., 2016). Nucleic acid detection tests including PCR have significant advantages over culture and serology in terms of sensitivity and speed, but nucleic acid amplification technologies still require specially trained personnel and sophisticated equipment (Mercante and Winchell, 2015). Therefore, the urinary antigen test is widely used worldwide (Mercante and Winchell, 2015; Cunha et al., 2016; Miyashita et al., 2016) because of the simplicity of the procedure and the rapid result.

The urinary antigen test for the diagnosis of *Legionella* pneumonia is considered to be a useful tool, but the sensitivity of this test for diagnosing *Legionella* pneumonia is reported to be in the range of 55–80% (Domínguez et al., 1999; Helbig et al., 2001; Yzerman et al., 2002; Helbig et al., 2003), which is not very high. Furthermore, it has the limitation that it cannot diagnose *Legionella* pneumonia due to non-*L. pneumophila* SG1 (Fields et al., 2002; Helbig et al., 2003). Previous studies have reported that 20–50% of *Legionella* pneumonia cases are caused by non-*L. pneumophila* SG1 (Benin et al., 2002; Helbig et al., 2002; Amemura-Maekawa et al., 2010). Since such patients can be underdiagnosed using only the urinary antigen test, there is a need for a urinary antigen test kit that can detect all species and SGs of *L. pneumophila* (Fields et al., 2002).

Asahi Kasei Pharma Corporation (Tokyo, Japan) has developed a novel urinary antigen test kit using immunochromatography that can diagnose *Legionella* pneumonia caused by all SGs of *L. pneumophila*. This novel kit contains the antibody for the L7/L12 ribosomal protein, which is specific to each bacterium, including *L. pneumophila* (Kolberg et al., 1997).

The aim of this study was to evaluate the diagnostic utility of the novel urinary antigen test kit for diagnosing *Legionella* pneumonia including all SGs of *L. pneumophila*, in comparison with existing urinary antigen test kits.

Methods

Study population

Pneumonia patients suspected by attending physicians to have disease caused by *Legionella* species based on symptoms, laboratory examinations, and radiological findings, and whose urine samples could be collected, were enrolled prospectively. Pneumonia patients who were not suspected to have *Legionella* pneumonia were also enrolled at the discretion of the attending physician. This study was conducted from April 2016 to December 2018 in 16 hospitals in Japan. Pneumonia was diagnosed using the methods outlined in a previous report (Mandell et al., 2007): patients who had abnormal shadows on radiological examinations, with at least one symptom of fever, cough, sputum, chest pain, or general malaise, as well as one of either abnormal findings on auscultation or an increased inflammatory reaction, were diagnosed with pneumonia. Patients who were <15 years old and those with hospital-acquired pneumonia were excluded. This study was registered in the UMIN clinical trials registry (UMIN000022298) and was approved by the institutional review boards of all hospitals. All patients gave their written, informed consent.

Study design

Three urinary antigen tests, including the novel LAC-116 kit (Asahi Kasei Pharma Corporation, Tokyo, Japan), BinaxNOW *Legionella* (Abbott Diagnostics Medical, Lake Forest, CA, USA) (Binax), and Q-line Kyokutou *Legionella* (Kyokutou Corporation, Tokyo, Japan) (Q-line), were used for all patients in all participating hospitals. Sputum culture using BCYE-α and WYO-α, nucleic acid detection tests by PCR or loop-mediated isothermal amplification (LAMP) of sputum, and serum antibody tests were performed at the discretion of the attending physician in daily clinical practice.

In addition, residual samples of urine, sputum, and serum that were obtained in daily clinical practice were stored frozen at below −20 °C in each hospital, and these samples were transferred to Asahi Kasei Pharma Corporation for urinary antigen testing, culture, nucleic acid detection tests including both PCR and LAMP, and serum antibody testing by Denka Seiken (Denka Seiken Corporation, Tokyo, Japan), as much as possible.

The following patient data were collected: age, sex, comorbidities, history of bathing in hot springs or public baths and of soil exposure, symptoms, vital signs, laboratory findings including blood tests, urinary antigen tests, and sputum culture, radiological findings, severity of pneumonia assessed by CURB-65 score (confusion, urea >7 mmol/l, respiratory rate ≥30 breaths per minute, low blood pressure (systolic <90 mmHg or diastolic ≤60 mmHg), and age ≥65 years) (Lim et al., 2003) and Pneumonia Severity Index (PSI) (Fine et al., 1997), antibiotic therapy, and outcomes.

The novel urinary antigen test kit for diagnosing Legionella *pneumonia due to all* Legionella pneumophila *serogroups*

Asahi Kasei Pharma Corporation developed a new kit for diagnosing *L. pneumophila* pneumonia caused by all SGs on May 11, 2015. This is a hybrid kit that can detect the lipopolysaccharide

in *L. pneumophila* SG1 and L7/L12 ribosomal protein in *L. pneumophila* SGs 1–15. L7/L12 was found to be one of the ribosomal proteins in *Escherichia coli* (Howe and Hershey, 1983). Using the antibody for L7/L12 ribosomal protein, Asahi Kasei Pharma Corporation first developed a point-of-care testing kit using immunochromatography that could diagnose lower respiratory tract infections caused by *Mycoplasma pneumoniae*, with sample collection by nasopharyngeal swab (Sano et al., 2016).

The test procedure for the new kit consists of the following four steps: (1) preparation of the sample diluent; (2) collection of 0.5 mL of the patient's urine using the dropper included; (3) mixing of the sample diluent and the patient's urine; and (4) applying five drops to the kit. The result of the test is evaluated within 15 min after sampling. If both the control and test lines turn reddish-purple, the result is considered positive. This new kit was able to detect *Legionella* species including *L. pneumophila* SGs 1–15, *L. dumoffii*, and *L. bozemanae* in the pre-developmental phase in internal studies performed by Asahi Kasei Pharma Corporation, although it could not detect other *Legionella* species including *L. longbeachae*, which is the second most common *Legionella* species (Whiley and Bentham, 2011).

Diagnosis of pneumonia due to Legionella *species*

Legionella pneumonia was diagnosed if at least one of the following was satisfied: (1) positive result of the urinary antigen test by Binax or Q-line; (2) *Legionella* species identified by sputum culture; (3) positive result of nucleic acid detection testing by both PCR and LAMP; or a (4) four-fold increase by paired serum antibody testing. In this study, *Legionella* pneumonia was not diagnosed in those cases with a positive result only with the new kit, because of the possibility of false-positives.

Study outcomes

The primary outcome was the diagnostic accuracy of the new kit for diagnosing *Legionella* pneumonia. The secondary outcome was the diagnostic correlations of the new kit for *Legionella* pneumonia with the existing urinary antigen test kits Binax and Q-line. Cases with discordant results between the new kit and the existing kits were also analyzed.

Statistical analysis

Continuous variables are expressed as the median and interquartile range, and categorical variables are expressed as the number and percentage. To determine the diagnostic accuracy of the new kit compared with the existing urinary antigen test kits, a cross-tabulation table was used to calculate the positive rate, negative rate, positive match rate, negative match rate, overall match rate, positive predictive value (PPV), and negative predictive value (NPV).

Results

Baseline characteristics of the patients

Of the 253 patients included, 56 were diagnosed with *Legionella* pneumonia. Figure 1 shows the diagnostic methods and numbers of patients diagnosed by each method. **Supplementary Material Table S1 report**s the number of patients for each diagnostic method for all patients, *Legionella* pneumonia patients, and non-*Legionella* pneumonia patients. In the non-*Legionella* pneumonia patient group, all patients were tested with the three urinary antigen tests, except for one patient who was tested with the new kit and Binax but not by Q-line. Overall, 93.9% of non-*Legionella* pneumonia patients were tested with two or more diagnostic methods including the urinary antigen tests, and 51.3% were tested with urinary antigen tests, sputum culture, and sputum nucleic acid detection tests.

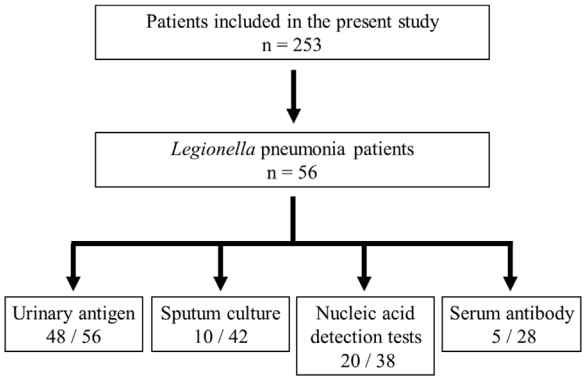

Figure 1. Study flow chart. For the 56 *Legionella* pneumonia patients, the number of positive results and the results of diagnostic tests including urinary antigen tests, sputum culture, nucleic acid detection tests, and serum antibody tests are shown.

The baseline clinical characteristics of all *Legionella* pneumonia patients are listed in Table 1. Their median age was 71 years, and 76.8% were male. The most common comorbidity was diabetes mellitus, followed by chronic heart disease, malignant disease, and chronic liver disease. Twenty patients (35.7%) had bathed in a hot spring or public bath and 10 patients (17.9%) had soil exposure. The most common symptom was fever (80.4%), followed by cough (46.4%). Digestive symptoms, including abdominal pain and diarrhea, were uncommon.

Table 2 shows the severity of pneumonia, antibiotic treatment, and outcome for all *Legionella* pneumonia patients. Overall, 18 (32.1%) patients had a CURB-65 score $\geq$3 points and 36 (64.3%) had a PSI class $\geq$ IV. The initial antibiotics were appropriate in 42 (75%) patients. Thirteen patients (23.2%) were admitted to the intensive care unit, but no patient died.

Diagnostic accuracy of each Legionella *urinary antigen test kit*

Table 3 shows the results of each *Legionella* urinary antigen test kit, including the new kit, Binax, and Q-line. The sensitivity of the new kit in all patients was 79%, which was slightly inferior to Binax (84%), but superior to Q-line (71%). If patients diagnosed only by Binax or Q-line were excluded, the sensitivity of the new kit was 68%, the same sensitivity as Binax (68%) and superior to Q-line (56%). In the patients with sputum sample cultures, *L. pneumophila* SG1 was the most common (6/10), followed by *L. pneumophila* SG undetected (2/10), *L. pneumophila* SG1 and SG5 (1/10), and *L. pneumophila* SG2 (1/10). The one patient with *L. pneumophila* SG2 was detected only by the new kit and not by Binax or Q-line.

Diagnostic correlations of the new kit with Binax and Q-line

Table 4 shows the cross-tabulations of the urinary antigen test kit results between the new kit and Binax and between the new kit and Q-line. The results for the overall match rate, positive match rate, negative match rate, PPV, and NPV between the new kit and Binax were 96.8% (244/252), 89.4% (42/47), 98.5% (202/205), 93.3% (42/45), and 97.6% (202/207), respectively. The results for the overall match rate, positive match rate, negative match rate, PPV, and NPV between the new kit and Q-line were 96.4% (243/252), 95.0% (38/40), 96.7% (205/212), 84.4% (38/45), and 99.0% (205/207), respectively.

Table 1
Baseline clinical characteristics of *Legionella* pneumonia patients.

	All patients *N* = 56
Age (years)	71 (61–79)
Male	43 (76.8)
Comorbidities	
Chronic obstructive pulmonary disease	2 (3.6)
Bronchial asthma	3 (5.4)
Diabetes mellitus	7 (12.5)
Chronic heart disease	5 (8.9)
Malignant disease	5 (8.9)
Chronic renal disease	3 (5.4)
Chronic liver disease	5 (8.9)
Cerebrovascular disease	4 (7.1)
History of bathing in hot spring or public bath	20 (35.7)
History of soil exposure	10 (17.9)
Symptoms	
Fever	45 (80.4)
Cough	26 (46.4)
Sputum	17 (30.4)
Dyspnea	19 (33.9)
Abdominal pain	2 (3.4)
Diarrhea	6 (10.7)
Arthralgia	6 (10.7)
Myalgia	4 (7.1)
Mental disturbance	16 (28.6)
Vital signs	
Temperature (°C)	39.0 (38.0–39.9)
Heart rate (/min)	96 (86–108)
Relative bradycardia	29 (51.8)
Systolic blood pressure (mmHg)	133 (119–147)
Laboratory findings	
C-reactive protein (mg/l)	221.5 (160.8–309.7)
Total protein (g/dl)	6.7 (6.1–7.1)
Albumin (g/dl)	3.1 (2.8–3.4)
Total bilirubin (mg/dl)	0.85 (0.60–1.20)
Aspartate aminotransferase (U/l)	56 (34–96)
Alanine aminotransferase (U/l)	32 (20–65)
Lactate dehydrogenase (U/l)	308 (236–394)
Blood urea nitrogen (mg/dl)	19 (13–24)
Creatinine (mg/dl)	1.01 (0.80–1.49)
Sodium (mmol/l)	134.5 (130.0–138.3)
White blood cells ($\times 10^3/\mu l$)	10.2 (7.8–13.2)
Hemoglobin (g/dl)	12.9 (11.4–13.7)
Platelets ($\times 10^4/\mu l$)	16.8 (13.2–22.0)

Data are shown as the number (%) or median (interquartile range).

Table 2
Severity of pneumonia, treatment, and outcomes of *Legionella* pneumonia patients.

	All patients *N* = 56
Severity of pneumonia	
CURB-65 (points)	
0	8 (14.3)
1	15 (26.8)
2	15 (26.8)
3	15 (26.8)
4	3 (5.4)
5	0 (0)
Pneumonia Severity Index (points)	100 (83–129)
Pneumonia Severity Index (class)	
I	1 (1.8)
II	9 (16.1)
III	10 (17.9)
IV	24 (42.9)
V	12 (21.4)
Intensive care unit admission	13 (23.2)
Mechanical ventilation support	10 (17.9)
Vasopressor drug use	6 (10.7)
Initial appropriate treatment	42 (75.0)
Levofloxacin	26 (46.4)
Ciprofloxacin	6 (10.7)
Pazufloxacin	2 (3.6)
Azithromycin	5 (8.9)
Levofloxacin + azithromycin	3 (5.4)
Initial inappropriate treatment	14 (25.0)
In-hospital mortality	0 (0)
30-day mortality	0 (0)

Data are shown as the number (%) or median (interquartile range). CURB-65, confusion, urea >7 mmol/l, respiratory rate ≥30 breaths/min, low blood pressure (systolic <90 mmHg or diastolic ≤60 mmHg), and age ≥65 years.

Cases with inconsistent results between the new kit and existing kits

Supplementary Material Table S2 shows the cases in which the results of the new kit and existing kits were discordant. Two patients (patients 7 and 8) had a positive result with the new kit, but showed negative results by Binax or Q-line. Of these, one patient (patient 7) was diagnosed with *Legionella* pneumonia due to *L. pneumophila* SG2 by sputum culture. The other patient (patient 8) may in fact have had *Legionella* pneumonia, although this patient's result was defined as false-positive. Patient 13 had a positive result only by Binax, but negative by all other tests including the new kit, Q-line, sputum culture, nucleic acid detection tests, and serum antibody. The Binax test was repeated using this patient's urine after boiling, and the result was negative. Therefore, based on a previous report (Doskeland and Berdal, 1980), this patient's result may have been a false-positive.

Discussion

This study showed that the new kit was useful for diagnosing *Legionella* pneumonia when compared with the existing urinary antigen test kits, Binax and Q-line. Regarding sensitivity, the new kit (79%) was slightly inferior to Binax (84%) but superior to Q-line (71%). The new kit also had a good diagnostic correlation with Binax and Q-line. Only the new kit was able to diagnose *Legionella* pneumonia due to *L. pneumophila* SG2, although there was only one patient confirmed to have a non-*L. pneumophila* SG1 in this study.

The weak point of the existing *Legionella* urinary antigen test kits is that they are not able to diagnose *Legionella* pneumonia due to non-*L. pneumophila* SG1 (Phin et al., 2014). *Legionella* pneumonia caused by non-*L. pneumophila* SG1 has been reported to account for 20–50% of cases worldwide (Benin et al., 2002; Helbig et al., 2002; Amemura-Maekawa et al., 2010), although rates differ in different areas. These *Legionella* pneumonia patients may therefore be underdiagnosed. Thus, early and appropriate diagnosis is important to improve their prognosis through adequate antibiotic therapy. In the present study, the new kit theoretically detected all SGs of *L. pneumophila.* It could detect *Legionella* pneumonia due to *L. pneumophila* SG2, even though Binax and Q-line could not. Therefore, the daily clinical use of this new kit is expected to diagnose *Legionella* pneumonia caused by non-*L. pneumophila* SG1 early and appropriately in the future. However, because there was only one *Legionella* pneumonia patient with non-*L. pneumophila* SG1 in the present study, future studies are needed to evaluate the usefulness of the new kit for diagnosing *Legionella* pneumonia due to non-*L. pneumophila* SG1 in many more patients.

The detectability of non-*L. pneumophila* SG1 *Legionella* pneumonia patients is novel, but the reliable diagnosis of *L. pneumophila* SG1 *Legionella* pneumonia patients is important, because most patients with *Legionella* pneumonia have *L. pneumophila* SG1 (Helbig et al., 2002; Amemura-Maekawa et al., 2010). In a systematic review, Shimada et al. reported that the sensitivity of the *Legionella* urinary antigen test kit was 77% (Shimada et al., 2009). The sensitivity of the new kit in the present study was 79%, similar to previous reports (Shimada et al., 2009), and it was comparable to Binax, which is thought to be widely used worldwide. Therefore, the new kit has the advantage of detecting non-*L. pneumophila* SG1 *Legionella* pneumonia patients without decreasing the diagnostic accuracy for *L. pneumophila* SG1

Table 3
Positive results of all *Legionella* urinary antigen test kits.

Urinary antigen test kit	All patient n = 56	Urinary antigen test n = 48	Sputum culture n = 10	Nucleic acid detection tests n = 20	Serum antibody n = 5	Culture or nucleic acid detection tests or serum antibody n = 25
LAC-116	44 (79)	43 (90)	10 (100)	13 (65)	4 (80)	17 (68)
BinaxNOW *Legionella*	47 (84)	47 (98)	9 (90)	13 (65)	4 (80)	17 (68)
Q-line Kyokutou *Legionella*	40 (71)	40 (83)	7 (70)	10 (50)	4 (80)	14 (56)

Data are shown as the number (%).

Table 4
Correlations between the new kit and existing kits.

LAC-116 vs Binax				
		Binax		Total
		Positive	Negative	
LAC-116	Positive	42	3	45
	Negative	5	202	207
Total		47	205	252
LAC-116 vs Q-line		Q-line		Total
		Positive	Negative	
LAC-116	Positive	38	7	45
	Negative	2	205	207
Total	40	212	252	Total

Binax, BinaxNOW *Legionella*; Q-line, Q-line Kyokutou *Legionella*.

Legionella pneumonia patients compared with existing *Legionella* urinary antigen tests.

Regarding the diagnostic methods in *Legionella* pneumonia, the urinary antigen test is the most widely used test (Shimada et al., 2009; Mercante and Winchell, 2015; Cunha et al., 2016; Miyashita et al., 2016) due to the simplicity of the procedure without the need for specialized equipment and the rapid availability of results compared to culture, nucleic acid detection tests, and serum antibody tests. However, culture of respiratory specimens is the gold standard for the diagnosis of *Legionella* pneumonia (Fields et al., 2002), and it is an important diagnostic method because it can identify all SGs of *L. pneumophila* and *Legionella* species other than *L. pneumophila*. Although the new kit could detect all SGs of *L. pneumophila*, it cannot identify the SG. Therefore, culture or nucleic acid detection tests of a respiratory specimen should also be performed to identify the SG of *L. pneumophila* as much as possible.

Of great importance, a suspicion of *Legionella* pneumonia is essential for reaching a diagnosis of *Legionella* pneumonia, because each of the examinations, including urinary antigen tests, sputum culture, nucleic acid detection tests, and serum antibody tests, cannot be performed if the attending physician does not suspect *Legionella* pneumonia. There are some scoring systems for predicting *Legionella* pneumonia (Cunha, 1998; Fiumefreddo et al., 2009; Miyashita et al., 2019). Among them, the scoring systems suggested by Fiumefreddo et al. (2009) and Miyashita et al. (2019) have good predictive performance (area under the curve, Fiumefreddo's score: 0.86, Miyashita's score: 0.93), and these systems are easy to use in daily clinical practice because they include only six items. However, we have previously reported that the scoring system of Fiumefreddo et al. was useful to predict *Legionella* pneumonia due to *L. pneumophila* SG1, but it was not useful for that due to non-*L. pneumophila* SG1 (Ito et al., 2017). In that study, if the cut-off was $\geq$2 points, most *Legionella* pneumonia cases due to *L. pneumophila* SG1 were identified (95.7%), but only about half of *Legionella* pneumonia cases due to non-*L. pneumophila* SG1 were identified (54.5%) (Ito et al., 2017). We also showed that almost all *Legionella* pneumonia cases caused by non-*L. pneumophila* SG1 had a lobar pneumonia pattern, including consolidation and ground-glass opacities (Ito et al., 2017). Yu et al. also reported that consolidation and ground-glass opacities were the main findings on computed tomography, and a non-segmental distribution was significantly more frequent than a segmental distribution in *Legionella* pneumonia (Yu et al., 2010). On the other hand, some previous studies have shown that multilobar or multisegmental, well-circumscribed air-space opacities intermingled with ground-glass opacities are typical computed tomography findings (Sakai et al., 2007; Mittal et al., 2017). Therefore, this novel kit should be used for patients with these radiological findings in order to diagnose many more *Legionella* pneumonia patients, even though the predictive score is low.

There are some limitations in the present study. First, *Legionella* pneumonia may have been underdiagnosed because only patients clinically suspected to have *Legionella* pneumonia by attending physicians were included. As mentioned above, some *Legionella* pneumonia patients with non-*L. pneumophila* SG1 had low predictive scores and were difficult to suspect; therefore, *Legionella* pneumonia due to non-*L. pneumophila* SG1 may have been underdiagnosed in the present study. Second, there was only one patient with *Legionella* pneumonia due to non-*L. pneumophila* SG1; therefore, whether the new kit is useful for diagnosing *Legionella* pneumonia caused by non-*L. pneumophila* SG1 is unclear. Future studies are needed to confirm the usefulness of the new kit in a much larger number of patients than in the present study. Third, the value of the new kit may be underestimated because cases with a positive result from only the new kit were not included in the present study. Indeed, there was one patient who had a positive result with the new kit and negative results with Binax and Q-line, and this patient may in fact have had *Legionella* pneumonia. Finally, the performance of the three urinary antigen tests including the new kit could have been overestimated, because there may have been some *Legionella* pneumonia patients in the non-*Legionella* pneumonia patient group. However, all patients underwent urinary antigen tests, and 93.9% of patients were tested with two or more diagnostic methods including urinary antigen tests in the non-*Legionella* pneumonia patient group. Therefore, microbiological examinations were assessed as much as possible, and the diagnostic accuracy of the three urinary antigen tests was reasonable.

A strength of the present study was that it was relatively large, including 253 patients, with 56 *Legionella* pneumonia patients, and it was conducted at 16 hospitals throughout Japan. The new kit has been available commercially as Ribotest Legionella since February 2019, and this novel kit can be used routinely in daily clinical practice. Although the results of this study could be applicable in other areas and countries, we believe that further studies are needed to evaluate the usefulness of the new kit in other countries.

In conclusion, the novel kit can theoretically diagnose *Legionella* pneumonia due to both *L. pneumophila* SG1 and non-SG1, and it is therefore useful for diagnosing *Legionella* pneumonia. In the future, early and appropriate diagnosis of *Legionella* pneumonia due to non-*L. pneumophila* SG1 can be expected with this novel kit. We believe that the use of this kit may improve the prognosis of *Legionella* pneumonia patients.

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Ethical approval and consent to participate

This study was registered with UMIN (UMIN000022298) and was approved by the institutional review boards of all participating hospitals. All patients gave their informed consent to participate in this study.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.10.106.

References

Amemura-Maekawa J, Kura F, Helbig JH, Chang B, Kaneko A, Watanabe Y, et al. Characterization of Legionella pneumophila isolates from patients in Japan according to serogroups, monoclonal antibody subgroups and sequence types. J Med Microbiol 2010;59:653–9.

Arancibia F, Cortes CP, Valdés M, Cerda J, Hernández A, Soto L, et al. Importance of Legionella pneumophila in the etiology of severe community-acquired pneumonia in Santiago, Chile. Chest 2014;145:290–6.

Benin AL, Benson RF, Besser RE. Trends in Legionnaires disease, 1980-1998: declining mortality and new patterns of diagnosis. Clin Infect Dis 2002;35:1039–46.

Burillo A, Pedro-Botet ML, Bouza E. Microbiology and epidemiology of Legionnaire's Disease. Infect Dis Clin North Am 2017;31:7–27.

Cillóniz C, Ewig S, Polverino E, Marcos MA, Esquinas C, Gabarrús A, et al. Microbial aetiology of community-acquired pneumonia and its relation to severity. Thorax 2011;66:340–6.

Cunha BA. Clinical features of Legionnaires' disease. Semin Respir Infect 1998;13:116–27.

Cunha BA, Burillo A, Bouza E. Legionnaires' disease. Lancet 2016;387:376–85.

Domínguez J, Galí N, Matas L, Pedroso P, Hernández A, Padilla E, et al. Evaluation of a rapid immunochromatographic assay for the detection of Legionella antigen in urine samples. Eur J Clin Microbiol Infect Dis 1999;18:896–8.

Doskeland SO, Berdal BP. Bacterial antigen detection in body fluids: methods for rapid antigen concentration and reduction of nonspecific reactions. J Clin Microbiol 1980;11:380–4.

Fields BS, Benson RF, Besser RE. Legionella and Legionnaires' disease: 25 years of investigation. Clin Microbiol Rev 2002;15:506–26.

Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE, et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med 1997;336:243–50.

Fiumefreddo R, Zaborsky R, Haeuptle J, Christ-Crain M, Trampuz A, Steffen I, et al. Clinical predictors for Legionella in patients presenting with community-acquired pneumonia to the emergency department. BMC Pulm Med 2009;19 (9):4.

Heath CH, Grove DI, Looke DF. Delay in appropriate therapy of Legionella pneumonia associated with increased mortality. Eur J Clin Microbiol Infect Dis 1996;15:286–90.

Helbig JH, Uldum SA, Lück PC, Harrison TG. Detection of Legionella pneumophila antigen in urine samples by the BinaxNOW immunochromatographic assay and comparison with both Binax Legionella Urinary Enzyme Immunoassay (EIA) and Biotest Legionella Urin Antigen EIA. J Med Microbiol 2001;50:509–16.

Helbig JH, Bernander S, Pastoris MC, Etienne J, Gaia V, Lauwers S, et al. Pan-European study on culture-proven Legionnaires' disease: distribution of Legionella pneumophila serogroups and monoclonal subgroups. Eur J Clin Microbiol Infect Dis 2002;21:710–6.

Helbig JH, Uldum SA, Bernander S, Lück PC, Wewalka G, Abraham B, et al. Clinical utility of urinary antigen detection for diagnosis of community-acquired, travel-associated, and nosocomial Legionnaires' disease. J Clin Microbiol 2003;41:838–40.

Howe JG, Hershey JW. Initiation factor and ribosome levels are coordinately controlled in Escherichia coli growing at different rates. J Biol Chem 1983;258:1954–9.

Ishida T, Hashimoto T, Arita M, Ito I, Osawa M. Etiology of community-acquired pneumonia in hospitalized patients: a 3-year prospective study in Japan. Chest 1998;114:1588–93.

Ishida T, Tachibana H, Ito A, Tanaka M, Tokioka F, Furuta K, et al. Clinical characteristics of severe community-acquired pneumonia among younger patients: an analysis of 18 years at a community hospital. J Infect Chemother 2014;20:471–6.

Ishiguro T, Takayanagi N, Yamaguchi S, Yamakawa H, Nakamoto K, Takaku Y, et al. Etiology and factors contributing to the severity and mortality of community-acquired pneumonia. Intern Med 2013;52:317–24.

Ito A, Ishida T, Washio Y, Yamazaki A, Tachibana H. Legionella pneumonia due to non-Legionella pneumophila serogroup 1: usefulness of the six-point scoring system. BMC Pulm Med 2017;17:211.

Kolberg J, Høiby EA, Lopez R, Sletten K. Monoclonal antibodies against Streptococcus pneumoniae detect epitopes on eubacterial ribosomal proteins L7/L12 and on streptococcal elongation factor Ts. Microbiology 1997;143:55–61.

Lim WS, Macfarlane JT, Boswell TC, Harrison TG, Rose D, Leinonen M, et al. Study of community acquired pneumonia aetiology (SCAPA) in adults admitted to hospital: implications for management guidelines. Thorax 2001;56:296–301.

Lim WS, van der Eerden MM, Laing R, Boersma WG, Karalus N, Town GI, et al. Defining community-acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax 2003;58:377–82.

Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007;44:S27–72.

Mercante JW, Winchell JM. Current and emerging Legionella diagnostics for laboratory and outbreak investigations. Clin Microbiol Rev 2015;28:95–133.

Mittal S, Singh AP, Gold M, Leung AN, Haramati LB, Katz DS. Thoracic imaging features of Legionnaire's disease. Infect Dis Clin North Am 2017;31:43–54.

Miyashita N, Aoki Y, Kikuchi T, Seki M, Tateda K, Higa F, et al. Questionnaire survey results on diagnosis and treatment of Legionella. Japanese Society of Chemotherapy Legionella Therapeutic Drug Evaluation Committee. Jpn J Chemother 2016;64:66–75 (in Japanese).

Miyashita N, Horita N, Higa F, Aoki Y, Kikuchi T, Seki M, et al. Validation of a diagnostic score model for the prediction of Legionella pneumophila pneumonia. J Infect Chemother 2019;25:407–12.

Phin N, Parry-Ford F, Harrison T, Stagg HR, Zhang N, Kumar K, et al. Epidemiology and clinical management of Legionnaires' Disease. Lancet Infect Dis 2014;14:1011–21.

Saito A, Kohno S, Matsushima T, Watanabe A, Oizumi K, Yamaguchi K, et al. Prospective multicenter study of the causative organisms of community-acquired pneumonia in adults in Japan. J Infect Chemother 2006;12:63–9.

Sakai F, Tokuda H, Goto H, Tateda K, Johkoh T, Nakamura H, et al. Computed tomographic features of Legionella pneumophila pneumonia in 38 cases. J Comput Assist Tomogr 2007;31:125–31.

Sano G, Itagaki T, Ishiwada N, Matsubara K, Iwata S, Nakamori Y, et al. Characterization and evaluation of a novel immunochromatographic assay for pharyngeal Mycoplasma pneumoniae ribosomal protein L7/L12 antigens. J Med Microbiol 2016;65:1105–10.

Shimada T, Noguchi Y, Jackson JL, Miyashita J, Hayashino Y, Kamiya T, et al. Systematic review and metaanalysis: urinary antigen tests for Legionellosis. Chest 2009;136:1576–85.

von Baum H, Ewig S, Marre R, Suttorp N, Gonschior S, Welte T, et al. Community-acquired Legionella pneumonia: new insights from the German competence network for community acquired pneumonia. Clin Infect Dis 2008;46:1356–64.

Whiley H, Bentham R. Legionella longbeachae and legionellosis. Emerg Infect Dis 2011;17:579–83.

Yu H, Higa F, Hibiya K, Furugen M, Sato Y, Shinzato T, et al. Computed tomographic features of 23 sporadic cases with Legionella pneumophila pneumonia. Eur J Radiol 2010;74:e73–8.

Yzerman EP, den Boer JW, Lettinga KD, Schellekens J, Dankert J, Peeters M. Sensitivity of three urinary antigen tests associated with clinical severity in a large outbreak of Legionnaires' disease in The Netherlands. J Clin Microbiol 2002;40:3232–6.