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Diagnostic Accuracy of PCR Alone and Compared to Urinary Antigen Testing for Detection of *Legionella* spp.: a Systematic Review

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The diagnosis of Legionnaires' disease (LD) is based on the isolation of *Legionella* spp., a 4-fold rise in antibodies, a positive urinary antigen (UA), or direct immunofluorescence tests. PCR is not accepted as a diagnostic tool for LD. This systematic review assesses the diagnostic accuracy of PCR in various clinical samples with a direct comparison versus UA. We included prospective or retrospective cohort and case-control studies. Studies were included if they used the Centers for Disease Control and Prevention consensus definition criteria of LD or a similar one, assessed only patients with clinical pneumonia, and reported data for all true-positive, false-positive, true-negative, and false-negative results. Two reviewers abstracted data independently. Risk of bias was assessed using Quadas-2. Summary sensitivity and specificity values were estimated using a bivariate model and reported with a 95% confidence interval (CI). Thirty-eight studies were included. A total of 653 patients had confirmed LD, and 3,593 patients had pneumonia due to other pathogens. The methodological quality of the studies as assessed by the Quadas-2 tool was poor to fair. The summary sensitivity and specificity values for diagnosis of LD in respiratory samples were 97.4% (95% CI, 91.1% to 99.2%) and 98.6% (95% CI, 97.4% to 99.3%), respectively. These results were mainly unchanged by any covariates tested and subgroup analysis. The diagnostic performance of PCR in respiratory samples was much better than that of UA. Compared to UA, PCR in respiratory samples (especially in sputum samples or swabs) revealed a significant advantage in sensitivity and an additional diagnosis of 18% to 30% of LD cases. The diagnostic performance of PCR in respiratory samples was excellent and preferable to that of the UA. Results were independent on the covariate tested. PCR in respiratory samples should be regarded as a valid tool for the diagnosis of LD.

Pneumonia caused by *Legionella* spp. (Legionnaires' disease [LD]) is a life-threatening pulmonary infection. The most common species causing clinical disease in humans is *Legionella pneumophila* (1). In addition to *L. pneumophila*, 19 species are documented as human pathogens on the basis of their isolation from clinical specimens (2). LD can affect people both in the community (3) and in the hospital and, in both settings, can occur in outbreaks (4, 5). The true incidence of LD is difficult to assess, because the bacterial etiology for community-acquired pneumonia (CAP) is generally not documented in clinical practice. LD cannot be differentiated clinically or radiographically from CAP caused by other bacterial pathogens (6). As *Legionella* spp. are obligatory intracellular bacteria, they are unaffected by beta-lactam antibiotics and require specific treatment with high-dose quinolones or macrolides (7). Treatment providing coverage against *Legionella* spp. has been shown to improve clinical success (8). Thus, early diagnosis of LD is important and can have an effect on both public health and management in hospitals (9, 10).

Conventional methods for the diagnosis of LD consist of culture, antigen detection in urine (i.e., urine antigen [UA]), serological testing, and direct fluorescent antibody (DFA) staining or immunohistochemistry (IHC). PCR-based methods for the diagnosis of *Legionella* spp. are usually based on conserved regions of rRNA sequences for amplification; these regions are not specific and, hence, can be used for detection of any *Legionella* subspecies. Real-time PCR methods, on the other hand, frequently use the macrophage infectivity potentiator gene (*MIP*) as a target for the specific detection of *L. pneumophila*; hence, they are used for the detection of *L. pneumophila* only. PCR enables specific amplification of minute amounts of *Legionella* DNA, provides results within a short time frame, and has the potential to detect infections caused by *Legionella* spp. We systematically reviewed all studies assessing PCR in clinical samples for the diagnosis of LD. We also compared and assessed the value of PCR compared to, and combined with, UA.

MATERIALS AND METHODS

Inclusion criteria. We included prospective or retrospective cohort studies and case-control studies. Participants (both cases and controls) were patients with pneumonia, either CAP or hospital acquired, as defined by radiological signs and clinical symptoms and signs (i.e., target condition). Case-control studies in which controls were healthy people were analyzed separately.

The index test was PCR for *Legionella* spp. performed on any clinical sample (sputum, bronchoalveolar lavage [BAL] sample, serum, urine, sterile fluids, and tissues). Analyses were made separately for each clinical sample. Any PCR test was acceptable, including standard PCR or real-time, nested, multiplex, or other PCR, and the test could target any *Legionella* spp. genes. We primarily used the sample taken at the time closest to

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TABLE 1 Study characteristics and the Quadas-2 risk of bias assessment and applicability criteria

Study	Location	Year start to end	Study design	No. of patients		Study population	Laboratory methods beside PCR used	Patient selection:		Index test:		Reference standard:		Flow and timing: risk of bias
				With LD	Without LD			Risk of bias	Concerns regarding applicability	Risk of bias	Concerns regarding applicability	Risk of bias	Concerns regarding applicability	
Alexiou-Daniel 1998 (14)	Thessaloniki, Greece	1992–1997	Case-control	24	10	Patients suffering from proven LD	Culture or UA method: not stated	High	Low	Unclear	High	Low	Low	Unclear
Benitez 2013 (15)	Atlanta, USA	Not stated	Case-control	15	6	Patients suffering from proven LD	Culture or UA method: not stated	High	High	Unclear	High	Low	Low	Unclear
Bernander 1997 (16)	Stockholm, Sweden	Not stated	Case-control	25	33	Patients suffering from proven LD and other CAP	Culture: BCYE cx, MYW, and BMPAcx[a] agars, UA method: not stated	High	Low	Unclear	Low	Low	Low	Low
Cloud 2000 (17)	Utah, USA	Not stated	Prospective cohort	31	181	Patients suspected of having pneumonia caused by *Legionella*spp.	Culture: BCYE agar, UA method: not stated	High	High	Unclear	Unclear	Unclear	Low	Unclear
Diederen 2007 (18)	Multicenter, The Netherlands	1995–2005	Case-control	68	36	Patients suffering from proven LD	Culture: not stated, UA method: Binax	High	Low	Unclear	Low	Low	Low	Low
Diederen 2008 (19)	Tilburg, The Netherlands	2002–2005	Retrospective cohort	37	112	Hospitalized patients with CAP	Culture: BCYE agar, UA method: not stated	Low	Low	Unclear	Unclear	Unclear	Low	Unclear
Diederen 2009 (20)	Tilburg, The Netherlands	1998–2000	Prospective cohort	11	230	Hospitalized patients with CAP	Culture: not stated,UA method: Binax	Low	Low	Unclear	Low	Unclear	Low	Low
Fard 2012 (21)	Tehran, Iran	2009–2010	Prospective cohort	4	258	Hospitalized patients with CAP	Culture: BCYE agar, UA method: not stated	Low	Low	Unclear	High	Low	Low	Unclear
Hayden 2001 (22)	Minnesota, USA	1979–1999	Case-control	9	10	Not stated	Culture: BCYE agar, UA method: not stated	High	Low	Unclear	High	Low	Low	Unclear
Helbig 1999 (23)	Dresden, Germany	Not stated	Prospective cohort	58	224	Not stated	Culture or UA method: not stated	Low	Low	Unclear	High	Low	Low	High
Herpers 2003 (24)	Bilthoven, The Netherlands	2001–2002	Case-control	17	23	Patients suffering from proven LD and other CAP	Culture: not stated, UA method: Binax	High	Low	Unclear	High	Low	Low	Unclear
Jaulhac 1992 (25)	Strasbourg, Lyon, France	Not stated	Case-control	12	56	Patients suspected of having pneumonia caused by *Legionella*spp.	Culture: BCYE agar, UA method: not stated	High	Low	Unclear	High	Low	Low	Unclear
Jin 2001 (26)	Beijing, China	1998–1999	Case-control	15	31	Patients suffering from proven LD	Culture: BCYE agar, UA method: not stated	High	Low	Unclear	Low	Unclear	Low	Low
Jonas 1995 (27)	Mainz, Germany	Not stated	Retrospective cohort	10	246	Patients from intensive care units or from the hematology department	Culture: BCYE agar, UA method: not stated	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Kessler 1993 (28)	Graz, Austria	Not stated	Prospective cohort	6	46	Hospitalized patients with CAP (atypical)	Culture or UA method: not stated	Unclear	Low	Unclear	Unclear	Unclear	Low	Unclear

Kim 2001 (29)	Seoul, Korea	1997–2000	Prospective cohort	6	425	Hospitalized patients with CAP	Culture or UA method: not stated	Low	Low	Unclear	High	Unclear	Low	High
Koide 2004 (31)	Okinawa, Japan	1997–1999	Case-control	6	17	Patients suffering from proven LD and other CAP	Culture: not stated, UA method: Binax, Biotest	High	Low	Unclear	High	Low	Low	High
Koide 2006 (30)	Okinawa, Japan	1993–2004	Case-control	33	25	Patients suffering from proven LD and other CAP	Culture: not stated, UA method: Binax, Biotest, Binax NOW	High	Low	Unclear	High	Low	Low	High
Lisby 1994 (32)	Herlev, Denmark	Not stated	Prospective cohort	2	86	Patients suspected of having pneumonia caused by *Legionella*spp.	Culture: BCYE agar, UA method: not stated	Low	Low	Low	Unclear	Low	Low	Unclear
Loens 2008 (33)	Wilrijk, Belgium	2000–2002	Prospective cohort	4	143	Hospitalized patients with CAP	Culture: not stated, UA method: Binax	Low	Low	Unclear	Unclear	Unclear	Low	Unclear
Matsiota-Bernard 1994 (34)	Garches, France	Not stated	Case-control	12	17	Hospitalized patients with CAP	Culture: BCYE agar, UA method: not stated	High	Low	Unclear	High	Low	Low	Unclear
Matsiota-Bernard 1997 (35)	Garches, France	Not stated	Case-control	41	10	Patients suffering from proven LD	Culture or UA method: not stated	High	Low	Unclear	High	Low	Low	Unclear
Maurin 2010 (36)	Grenoble, France	2004–2006	Prospective cohort	19	201	Hospitalized patients with CAP	Culture: BCYE agar, UA method: not stated	Low	Low	Unclear	Unclear	Unclear	Low	Unclear
Mérault 2011 (37)	Multicenter, France	2007–2010	Case-control	22	74	Patients suffering from proven LD and other CAP	Culture: BCYE agar, UA method: Binax	High	Low	Unclear	High	Low	Low	Low
Miyashita 2004 (38)	Multicenter, Japan	1999–2000	Prospective cohort	8	200	Patients who were participants in a multicenter CAP surveillance study	Culture: BCYE agar, UA method: not stated	Low	Low	Unclear	Unclear	Unclear	Low	Unclear
Murdoch 1996 (39)	Canterbury, New Zealand	1992–1995	Case-control	28	24	CAP and nosocomial pneumonia surveillance studies	Culture: BCYE agar, UA method: not stated	High	Unclear	Unclear	Low	Low	Low	Low
Nomanpour 2012 (40)	Tehran, Iran	2009–2010	Prospective cohort	9	120	Hospitalized patients with CAP	Culture: BCYE agar, UA method: not stated	Low	Low	Unclear	Unclear	Unclear	Low	Unclear
Raggam 2002 (41)	Graz, Austria	Not stated	Prospective cohort	3	58	Patients suspected of having pneumonia caused by *Legionella*spp.	Culture: BCYE agar, UA method: not stated	Unclear	Unclear	Unclear	Unclear	Unclear	Low	Unclear
Ramirez 1996 (42)	Louisville, USA	Not stated	Prospective cohort	6	149	Hospitalized patients with CAP or nosocomial pneumonia	Culture: BCYE agar, UA method: not stated	Unclear	High	Unclear	Unclear	Unclear	Low	Low

(Continued on following page)

TABLE 1 (Continued)

Study	Location	Year start to end	Study design	No. of patients		Study population	Laboratory methods beside PCR used	Patient selection:		Index test:		Reference standard:		Flow and timing: risk of bias
				With LD	Without LD			Risk of bias	Concerns regarding applicability	Risk of bias	Concerns regarding applicability	Risk of bias	Concerns regarding applicability	
Rantakokko-Jalava 2001 (43)	Turku, Finland	Not stated	Prospective cohort	2	64	Hospitalized patients with CAP	Culture: BCYE agar, UA method: not stated	Low	Low	Unclear	Unclear	Unclear	Low	Unclear
Reischl 2002 (44)	Regensburg, Germany	Not stated	Case-control	26	39	Not stated	Culture: BCYE agar, UA method: not stated	High	Unclear	Unclear	High	Low	Low	Unclear
Socan 2000 (45)	LjubOana, Slovenia	Not stated	Prospective cohort	22	60	Hospitalized patients with CAP	Culture or UA method: not stated	Unclear	Low	Unclear	Low	Unclear	High	Low
Templeton 2003 (46)	Antwerp, Belgium	Not stated	Prospective cohort	4	72	Patients suffering from proven LD (clinical outbreak of LD)	Culture: BCYE agar, UA method: not stated	Unclear	Low	Unclear	Unclear	Low	Low	Unclear
van de Veerdonk 2009 (47)	Hertogenbosch, The Netherlands	Not stated	Case-control	11	20	Patients suffering from proven LD	Culture: not stated, UA method: Binax NOW	High	Unclear	Unclear	Low	Low	Low	Low
Weir 1998 (48)	Meryland, USA	1996–1996	Prospective cohort	4	122	Hospitalized patients with CAP	Culture: BCYE agar, UA method: not stated	Unclear	Low	Unclear	Unclear	Unclear	Low	High
Welti 2003 (49)	Lausanne, Zurich, Switzerland	2001–2001	Prospective cohort	11	27	Hospitalized patients with CAP	Culture or UA method: not stated	Unclear	Low	Unclear	Unclear	Unclear	Low	Unclear
Wilson 2003 (50)	Regensburg, Germany	Not stated	Case-control	7	41	Not stated	Culture: BCYE agar, UA method: not stated	High	Unclear	Unclear	High	Low	Low	Unclear
Yang 2009 (51)	Atlanta, USA	Not stated	Retrospective cohort	37	97	Patients suspected of having pneumonia caused by *Legionella spp.*	Culture: BCYE agar, UA method: not stated	Low	Low	Unclear	Unclear	Low	Low	Unclear

[a] BCYE, buffered charcoal yeast extract; cx, culture.

the onset of infection. If data were available for more than one test in a study, all results were extracted. We also extracted data on UA in studies reporting both tests separately and together with PCR results. The target condition was pneumonia (either community or hospital acquired). The reference standard included two levels of certainty (11).

We considered a culture positive for *Legionella* spp. when a 4-fold increase in serum antibodies for *Legionella* spp. occurred if taken 4 to 6 weeks after the clinical episode or when a positive UA confirmed infection. Diagnosis by antigen staining in respiratory secretion lung tissue or in pleural fluid by DFA staining or IHC was regarded as suspected infection (11). We considered all other cases as having no evidence for *Legionella* infection. These considerations are compatible with the Centers for Disease Control and Prevention (CDC) publication for preferred diagnostic tests for defining *Legionella* infection (11).

Electronic searches. We searched MEDLINE, LILACS, and KoreaMed databases without date or language restrictions, from inception to October 2014, using the following terms and their medical subject headings [MESH] (adapted for each database): (PCR or real-time or RT-PCR or reverse-transcription or nested-PCR or PCR) and (legionell* or legionair* or legionella[MESH] or Legionnaires' Disease[MESH]). In addition, we searched the European Conference of Clinical Microbiology and Infectious Diseases and the Interscience Conference on Antimicrobial Agents and Chemotherapy between the years 2010 and 2014 using only the key words for *Legionella* or Legionnaires' and PCR. We scanned the references of all included studies and reviews cited in the included studies.

Data collection and risk of bias assessment. Two reviewers independently selected studies for inclusion and extracted all data from the studies. Risk of bias assessment was conducted using the Quadas-2 tool (12).

Statistical analysis and data synthesis. We listed the number of true positives, true negatives, false positives, and false negatives per study, specimen, index test, primer gene used for PCR, and reference standard. We calculated the sensitivity and specificity values and the diagnostic odds ratio (DOR). We used the bivariate model for the data summary. Parameter estimates from the model were used to obtain hierarchical summary receiver operating curves, with 95% confidence intervals (CIs) and a 95% prediction region. We assessed the effect of the following covariates on results through subgroups analyses: PCR method, study design, primer gene used for PCR, number of LD cases, and the Quadas-2 domains. We compared the test performance of UA and PCR with that of UA alone in LD cases that were not diagnosed by UA alone, compared to the situation where either PCR or UA positivity defined a positive test result. Only direct test comparisons were performed. Studies were included only once in the analysis. Analyses were conducted using Stata 12 and RevMan 5.3 (13).

RESULTS

The search identified 804 references, of which 77 were selected for full-text review (see Fig. S1 in the supplemental material). Thirty-nine studies were excluded. A total of 38 studies that were published between the years 1993 and 2013 were included (14-51). Seven studies reported on the results of PCR in blood or serum, 4 trials reported on PCR in urine, 29 trials reported on PCR in BAL fluid or sputum, 3 trials reported on PCR in pharyngeal swabs, and 3 trials reported on PCR in lung tissue specimens. Five studies performed PCR in several sample types. Thirteen studies reported result for UA separately from results for PCR. Seventeen studies were case-control studies, 3 studies were retrospective cohorts, and the remaining 18 studies were prospective cohort studies. Altogether, 653 patients with confirmed LD, 8 patients with probable LD, 3,593 patients with pneumonia caused by pathogens other than *Legionella* spp., and 296 healthy control patients were included.

Data from prospective cohorts (not all patients underwent UA and/or culture) showed that cultures were positive in 164 of 2,562 patients with pneumonia (6.4%; 95% CI, 1.4% to 15.7%), UA was positive in 113 patients of 1,445 with pneumonia (7.82%; 95% CI, 2.2% to 15.2%), and PCR was positive in 309 of 3,463 patients with pneumonia (8.9%; 95% CI, 4.5% to 20.2%). Mortality was reported in 4 studies (weighted mean, 5.6%; range, 9.1% to 50%). Other study characteristics are presented in Table 1.

Risk of bias assessment. The Quadas-2 risk of bias assessment and applicability criteria are shown in Table 1. Only 12 of 38 studies were at low risk of bias regarding patient selection; 17 of 38 studies were at high risk (all of them were retrospective case-control studies). The remaining 9 of 38 were of unclear risk; among them, 3 studies were also of high risk of bias regarding the applicability of the selected population to this review. Concerns regarding the applicability of the index test were present in 15 of 38 studies and unclear in another 16 of 38 studies. High risk of bias regarding the flow chart, timing of the index test, and ensuring that all patients received the same tests were present in 5 studies, unclear in 24 studies, and at low risk of bias in 9 studies. Four of 38 studies were from developing nations. In 9 studies, clinical and radiological definitions for pneumonia were presented.

PCR technique. Details of the PCR techniques are presented Table 2. Standard PCR was used in 12 studies; real-time PCR, in 16 studies; real-time with multiplex PCR, in 4; and nested PCR, in 6 (in 2 studies (43, 49), 2 methods of PCR were used). Eight studies used primers targeting the *MIP* gene, 7 studies used both 5s rRNA and *MIP* genes, 6 studies used both 16S rRNA and *MIP* genes, 7 studies used the 5s rRNA gene, 7 studies used the 16S rRNA gene primers, and 4 studies used other genes (multiple genes were used in 4 studies). The primers targeted specifically *L. pneumophila* in 25 of 38 studies. DNA extraction was performed by the use of QIAamp kit ($n = 10$), MagNA Pure LC DNA isolation kit ($n = 5$), other commercial kits ($n = 10$), and phenol-chloroform protocols ($n = 13$). Internal/inhibition controls were described in 27 of 38 studies, and contamination/digestion controls were described in 19 of 38 studies.

Performance of PCR. Details of PCR sensitivity and specificity for the diagnosis of LD are presented in Table 3. The specificity was very high regardless of the sample; however, the sensitivity of urine and serum samples were very low (49.7% [95% CI, 26.5% to 73.0%] and 48.9% [95% CI, 38.4% to 59.5%], respectively). Respiratory samples had a high sensitivity for the detection of *Legionella* spp. by PCR. The summary sensitivity and specificity values of the bivariate model for all respiratory samples (BAL fluid, sputum, pharyngeal swabs, and tissue biopsies) were 97.4% (95% CI, 91.1% to 99.2%) and 98.6% (95% CI, 97.4% to 99.3%), respectively. The DOR was 2,826 (95% CI, 738 to 10,815).

Subgroup analysis based on sample type revealed summary sensitivity and specificity values of 97.7% (95% CI, 91.6% to 99.4%) and 98.6% (95% CI, 97.3% to 99.2%), respectively, for BAL fluid (combined occasionally with sputum) and 96.8% (95% CI, 41.2% to 99.9%) and 99.4% (95% CI, 91.7% to 99.9%), respectively, for sputum. Analysis based on studies with high methodological qualities ($n = 9$) yielded summary sensitivity and specificity values of 98.6% (95% CI, 57.7% to 99.9%) and 99.0% (95% CI, 96.9% to 99.9%), respectively.

There were no statistically significant differences in the performance of real-time, nested, and other PCR types. The use of inhibition control or contamination control did not affect significantly the performance of PCR. The use of genes specific to *L. pneumophila* was associated with increased sensitivity com-

TABLE 2 PCR methods

Study	PCR method	Volume used for PCR	DNA extraction method	No. of cycles	Primer gene	Internal/inhibition control	Contamination control	*Legionella* spp. detected by primer	Sample type	Time to sampling
Alexiou-Daniel 1998 (14)	Standard and hybrid	0.3 ml	Lysis buffer	40	16S rRNA	Not stated	Not stated	Various *Legionella*spp.	Serum	Not stated
Benitez 2013 (15)	Real-time	Not stated	MagNA Pure	45	*ssrA*, *MIP*, *WZM*	Yes	Not stated	*L. pneumophila*	Respiratory samples	Not stated
Bernander 1997 (16)	Nested PCR	0.25 ml	QIAamp	30	*MIP*	Not stated	Yes	*L. pneumophila*	Respiratory samples	2–7 days
Cloud 2000 (17)	standard	1 ml	QIAamp	38	16S rRNA	Not stated	Not stated	*L. pneumophila*	Respiratory samples	Not stated
Diederen 2007 (18)	Real-time	0.2 ml	MagNA Pure LC	50	5s rRNA, 16S rRNA, and *MIP*	Yes	Not stated	*L. pneumophila*	Serum	0 days
Diederen 2008 (19)	Real-time	0.2 ml	Total nucleic acid isolation kit	50	16S rRNA and *MIP*	Yes	Yes	Various *Legionella*spp.	Respiratory samples	Not stated
Diederen 2009 (20)	Real-time with multiplex	1 swab	MagNA Pure LC	50	16S rRNA and *MIP*	Yes	Not stated	*L. pneumophila*	Swab	0 days
Fard 2012 (21)	Real-time	1 ml	Lysis buffer	40	*MIP*	Yes	Yes	*L. pneumophila*	Respiratory samples	Not stated
Hayden 2001 (22)	Real-time	Not stated	Chelex 100	50	5s rRNA and *MIP*	Yes	Yes	Various *Legionella*spp.	Respiratory samples	Not stated
Helbig 1999 (23)	Standard	0.35 ml	Geneclean II kit	35	5s rRNA	Yes	Not stated	Various *Legionella*spp.	Urine	3–4 days
Herpers 2003 (24)	Real-time	Not stated	QIAamp	50	5S rRNA	Yes	Not stated	*L. pneumophila*	Respiratory samples	Not stated
Jaulhac 1992 (25)	Standard	2 ml	Lysis buffer	40	*MIP*	Not stated	Yes	*L. pneumophila*	Respiratory samples	Not stated
Jin 2001 (26)	Nested PCR	0.25 ml	Lysis buffer	35	16S rRNA and *MIP*	Not stated	Not stated	Various *Legionella*spp.	Respiratory samples	Beginning of hospital stay
Jonas 1995 (27)	Standard and hybrid	Not stated	QIAamp	40	16S rRNA	Not stated	Not stated	*L. pneumophila*	Respiratory samples	Not stated
Kessler 1993 (28)	Standard	0.5 ml	Lysis buffer	30	5s rRNA and *MIP*	Yes	Not stated	*L. pneumophila*	Respiratory samples	Not stated
Kim 2001 (29)	Standard	0.3 ml	Lysis buffer	35	5s rRNA	Not stated	Yes	Various *Legionella*spp.	Respiratory samples	1–100 days
Koide 2004 (31)	Standard	1 ml	Lysis buffer	35	5S rRNA	Yes	Yes	Various *Legionella*spp.	Serum, urine, and respiratory samples	5–247 days
Koide 2006 (30)	Standard	Not stated	Lysis buffer	53	5S rRNA	Yes	Yes	Various *Legionella*spp.	Serum, urine, and respiratory samples	Not stated
Lisby 1994 (32)	Standard	0.25 ml	Lysis buffer	40	16S rRNA	Yes	Not stated	*L. pneumophila*	Respiratory samples	Not stated
Loens 2008 (33)	Real-time	Not stated	QIAamp	45	*MIP*	Yes	Yes	*L. pneumophila*	Respiratory samples and swab	Not stated

Matsiota-Bernard 1994 (34)	Standard and hybrid	1 ml	Lysis buffer	30	5s rRNA and *MIP*	Yes	Not stated	*L. pneumophila*	Respiratory samples	Not stated
Matsiota-Bernard 1997 (35)	Standard and hybrid	Not stated	Lysis buffer	Not stated	5s rRNA and *MIP*	Yes	Not stated	*L. pneumophila*	Serum	2–15 days
Maurin 2010 (36)	Real-time	Not stated	QIAamp	Not stated	16S rRNA and *MIP*	Yes	Yes	*L. pneumophila*	Respiratory samples	Not stated
Mérault 2011 (37)	Real-time	0.2 ml	MagNA Pure LC	50	*LPS* gene cluster	Yes	Not stated	*L. pneumophila*	Respiratory samples	Not stated
Miyashita 2004 (38)	Real-time with multiplex	1 swab	QIAamp	40	Major outer membrane protein porin	Yes	Not stated	*L. pneumophila*	Swab	Not stated
Murdoch 1996 (39)	Standard	0.1–0.3 ml	Trizol	35	5S rRNA gene	Not stated	Yes	Various *Legionella*spp.	Serum and urine	1–30 days
Nomanpour 2012 (40)	Real-time with multiplex	Not stated	Lysis buffer	35	MIP	Yes	Yes	*L. pneumophila*	Respiratory samples and swab	Not stated
Raggam 2002 (41)	Real-time	0.1 ml	MagNA Pure LC	55	16S rRNA	Yes	Yes	Various *Legionella*spp.	Respiratory samples	Not stated
Ramirez 1996 (42)	Standard	1 swab	Lysis buffer	40	5s rRNA	Not stated	Yes	Various *Legionella*spp.	Swab	Not stated
Rantakokko - Jalava 2001 (43)	Real-time	0.2 ml	High pure PCR template preparation kit	45	16S rRNA	Yes	Yes	*L. pneumophila*	Respiratory samples	Not stated
Reischl 2002 (44)	Real-time	0.5 ml	High pure PCR template preparation kit	50	16S rRNA	Yes	Yes	*L. pneumophila*	Respiratory samples	Not stated
Socan 2000 (45)	Standard	0.2 ml	QIAamp	30	5s rRNA and *MIP*	Yes	Not stated	*L. pneumophila*	Urine	Beginning of hospital stay
Templeton 2003 (46)	Real-time	0.2 ml	High pure PCR template preparation kit	50	16S rRNA and *MIP*	Yes	Yes	Various *Legionella*spp.	Respiratory samples and swab	Not stated
van de Veerdonk 2009 (47)	Real-time	0.2 ml	NucliSens easyMAG	45	*MIP*	Yes	Not stated	*L. pneumophila*	Serum	0 days
Weir 1998 (48)	Standard	0.5 ml	Lysis buffer	Not stated	5s rRNA and *MIP*	Yes	Not stated	Various *Legionella*spp.	Respiratory samples	Not stated
Welti 2003 (49)	Real-time with multiplex	1 ml	QIAamp	50	16S rRNA and *MIP*	Yes	Yes	*L. pneumophila*	Respiratory samples	Not stated
Wilson 2003 (50)	Real-time	0.1 ml	QIAamp	45	*MIP*	Yes	Yes	*L. pneumophila*	Respiratory samples	Not stated
Yang 2009 (51)	Real-time	Not stated	KingFisher ML instrument and InviMag kit	Not stated	5s rRNA and 23S rRNA	Not stated	Not stated	*L. pneumophila*	Serum, respiratory samples and swab	Not stated

TABLE 3 Sensitivity and specificity of PCR in the diagnosis of Legionnaires' disease

Comparison, no. of studies	Sensitivity, % (95% CI[a])	Specificity, % (95% CI)	DOR[b] (95% CI)
PCR in urine samples, 5	49.7 (26.5–73.0)	98.2 (85.6–99.8)	54 (5.7–509)
PCR in blood samples, 7	48.9 (38.4–59.5)	99.8 (59.1–99.9)	889 (1.25–633,052)
PCR in respiratory samples			
All, 35	97.4 (91.1–99.2)	98.6 (97.4–99.3)	2,826 (738–10,815)
BAL[c] sample, 29	97.7 (91.6–99.4)	98.6 (97.3–99.2)	3,072 (733–12,786)
Sputum, 9	96.8 (41.2–99.9)	99.4 (91.7–99.9)	5,774 (30–1,110,511)
PCR in respiratory samples			
Retrospective studies excluded, 14	99.1 (63.3–99.9)	98.5 (97.1–99.2)	8,335 (127–546,320)
All high ROB[d] studies excluded, 9	98.4 (57.7–99.9)	99.0 (96.9–99.6)	6,447 (132–314,848)
Standard PCR, 8	98.8 (47.5–99.9)	97.9 (94.9–99.2)	3,996 (46–346,416)
Nested/hybrid PCR, 5	97.0 (83.4–99.5)	98.4 (92.9–99.6)	2,169 (227–20,697)
Real-time PCR, 17	97.9 (89.1–99.6)	98.7 (96.8–99.4)	3,675 (529–25,509)
L. pneumophila genes, 20	98.4 (91.4–99.7)	98.3 (96.7–99.2)	3,957 (655–23,875)
Various *Legionella*spp. genes, 9	95.7 (69.4–99.5)	99.1 (96.4–99.8)	2,680 (141–50,611)
No inhibition control, 9	97.0 (73.6–99.7)	98.0 (94.2–99.3)	1,720 (185–15,930)
Inhibition control, 20	98.3 (90.6–99.7)	98.6 (97.1–99.3)	4,435 (622–31,632)

[a] CI, confidence interval.
[b] DOR, diagnostic odds ratio.
[c] BAL, bronchoalveolar lavage.
[d] ROB, risk of bias.

pared to primers from genes that targeted various *Legionella* spp. (Table 3).

Comparison of PCR to UA. Details of the direct comparison of PCR and UA are detailed in Table 4. The summary sensitivity and specificity values of the bivariate model for UA in all studies were 77.0% (95% CI, 55% to 90.0%) and 100% (by definition), respectively. The DOR was 7,540 (95% CI, 289 to 19,652). In the direct comparison of PCR in respiratory secretions versus the use of UA, PCR had higher sensitivity ($P = 0.001$).

A subgroup analysis of cases of LD, when *a priori* excluding all cases of LD that were diagnosed by UA alone, yielded a summary sensitivity of 93.1% (95% CI, 63.9% to 99.0%) for PCR and 51.8% (95% CI, 33.1% to 69.1%) for UA.

Taking into account that UA is easily performed and available for each patient, while performing BAL fluid is invasive, contains certain risks, and is not readily available in all settings, we examined the performance of UA in sputum samples and/or pharyngeal swabs alone. The summary sensitivity and specificity values of PCR in sputum samples were 97.1% (95% CI, 59.6% to 99.8%) and 99.7% (95% CI, 91.4% to 99.9%), respectively; those of UA were 52.9% (95% CI, 30.8% to 73.9%) and 100% (by definition), respectively; those of either UA or PCR were 99.9% (95% CI, 99.9% to 99.9%) and 99.7% (95% CI, 90.2% to 99.9%), in 5 studies. In absolute terms, 11 of 61 patients (18%) with LD had a negative UA and a positive sputum PCR and would had been misdiagnosed by conventional methods.

DISCUSSION

We examined the accuracy of PCR alone and in comparison with UA in various clinical samples for the diagnosis of LD among patients with pneumonia, where the reference standard was proven or probable LD according to criteria suggested by the CDC (11). We demonstrated near perfect specificity values for all sample types and equally high sensitivity values for all respiratory samples (consisting of BAL fluid, sputum, pharyngeal swabs, tissue biopsy specimens, and other respiratory fluids). Overall, in 35 included studies that used any respiratory sample, the summary sensitivity and specificity estimates were 97.4% and 98.6%, re-

TABLE 4 Direct comparisons of PCR in respiratory samples versus UA[a]

Comparison, no. of studies	Sensitivity, % (95% CI[b])	Specificity, % (95% CI)	DOR[c] (95% CI)
UA: all studies, 13	77.0 (55.3–90.0)	99.9 (99.9–99.9)	7,540 (289–196,522)
PCR in respiratory samples vs UA[d]			
PCR, 8	93.1 (63.9–99.0)	99.1 (98.0–99.5)	1,515 (185–12,344)
UA, 8	51.8 (33.6–69.6)	99.9 (99.9–99.9)	NA[e]
UA or PCR, 8	95.6 (68.2–99.5)	99.1 (97.6–99.6)	2,577 (209–31,650)
PCR in sputum samples/swabs vs UA[d]			
PCR, 5	97.1 (59.6–99.8)	99.7 (91.4–99.9)	12,467 (171–907,125)
UA, 5	52.9 (30.8–73.9)	99.9 (99.9–99.9)	NA
UA or PCR, 5	99.9 (99.9–99.9)	99.7 (90.2–99.9)	NA

[a] UA, urinary antigen.
[b] CI, confidence interval.
[c] DOR, diagnostic odds ratio.
[d] All cases of LD diagnosed by UA alone were excluded.
[e] NA, not applicable.

spectively. In studies that used easy-to-obtain samples, such as sputum samples and pharyngeal swabs, the summary sensitivity and specificity estimates were 94.5% and 99.2%, respectively (13 studies). PCR sensitivity of urine and blood samples was low (roughly, 50%), rendering these samples unusable for clinical practice. We explored further the accuracy of PCR through subgroup and sensitivity analyses. We discovered that PCR sensitivity in respiratory samples remains very high after consideration for methodological quality, study design, and various PCR methods.

When we compared the results of PCR in respiratory samples to those of UA, we demonstrated improved sensitivity with similar specificity, regardless of the sample type. Furthermore, when cases that were diagnosed only by UA (without positive culture, serology, or DFA) and all cases that were diagnosed by BAL fluid were excluded, leaving a real-life comparison of PCR of pharyngeal swabs and/or sputum samples and the UA, PCR was considerably more sensitive than the UA and resulted in reclassification of 18% of patients with pneumonia and negative UA to an LD diagnosis.

Using the pooled sensitivity and specificity estimates of our review, the negative and positive predictive values (NPV and PPV, respectively) of the test can be calculated, using a defined prevalence of disease (52). With a prevalence of LD of 7.5% among patients with CAP (as observed from the prospective cohort studies in our review), negative PCR in respiratory sample excludes LD in 99.7% of patients, and positive PCR confirms LD in 84.9%. When both PCR on sputum sample/swab and UA are performed and either positive result defines a positive test, the NPV is 99.9%, and the PPV is 96%. Thus, a negative PCR rules out the diagnosis of LD with a very high probability (≥97%). Performing both tests increases the probability of ruling in LD without affecting specificity.

When LD is diagnosed, combination therapy directed at *Legionella* spp. increases the chances of survival (53) Therefore, the diagnosis of LD among patients hospitalized with CAP, especially when severe, may directly influence prognosis, while other patients may be treated with beta-lactam monotherapy (54). The diagnosis of LD today is based on several traditional methods. Culture requires special media, processing, and technical expertise, and 3 to 5 days are required to obtain a positive result. Serological testing for *Legionella* has little impact on clinical practice, as 20% to 30% of patients with LD do not develop a detectable antibody response if tested too early (55) or at all (56). The most common method currently used for diagnosing LD in the clinical setting is UA detection of *L. pneumophila* serogroup 1 (57). In a previous systematic review, the pooled sensitivity of UA assays for the detection of *L. pneumophila* serogroup 1 was 74% (95% CI, 68% to 81%), with a pooled specificity of 99% (95% CI, 98% to 99%) (58). Our results are in concordance with this systematic review (pooled UA sensitivity of 77% and near 100% specificity). However, the antigen is excreted in urine for weeks (and up to a year) after an infectious episode, which weakens its specificity (59). Furthermore, *L. pneumophila* serogroup 1 is the predominant *Legionella* spp. that causes LD in the United States and Europe but not in Asia and Australia (60). LD from non-*pneumophila Legionella* species is more common in immunocompromised patients, and *L. pneumophila* serogroups other than serogroup 1 can cause nosocomial outbreaks of LD (61, 62). In such cases, the UA might provide false-negative results. Diagnosis is LD among immunocompromised patients and in the nosocomial setting is critical, and PCR might improve the diagnosis of these cases significantly.

One of the main criticisms against the use of PCR in the diagnosis of LD, and one of the major limitations of analyzing PCR-based methods, is the lack of standardization in performance and reporting of the PCR methods. The contamination of commercial DNA extraction kits may produce false-positive results with the lack of a negative control (63). The occurrence of false-positive testing demonstrates the need for a standardized laboratory protocol for the needed stringent quality control requirements. Variable methods of sampling, extraction, and amplification protocols were used in the studies included in our review. We did not observe an effect of each parameter on results, except for improved sensitivity with primers made from a gene sequence of *L. pneumophila*. However, the number of studies included in our review was too small, and reporting was insufficient to assess individually and in combination the large number of variables relating to PCR methods. Moreover, PCR kits are expensive, PCR requires a dedicated laboratory equipment and personnel, and PCR is not easily interpreted, whereas the UA is relatively inexpensive (around $10 per test in the United States) and requires no special equipment or training.

In summary, we show an excellent sensitivity and specificity of PCR for the diagnosis of LD in any respiratory sample. The NPV given the usual disease prevalence was over 95% regardless of the subgroup examined. The PPV was also above 95%, thus making the PCR an excellent tool for ruling in or out LD. The sensitivity of the PCR in respiratory samples was superior to the UA and may result in the additional diagnosis of patients with *L. pneumophila* serogroup 1 LD and those with non-*pneumophila Legionella* species or non-serogroup 1 LD. We suggest using the PCR especially when infectdion with non-*pneumophila Legionella* species is possible.

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