Accuracy of diagnostic tests for Legionnaires’ disease: a systematic review

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Abstract

Purpose. Rapid and effective diagnosis of Legionnaires’ disease (LD) cases is extremely important so that timely and appropriate therapy can be provided, thereby lowering the morbidity and mortality rates and reducing the health and economic costs associated with this disease.

Methodology. Diagnosis is established solely by microbiological tests. There are several methods available, each with different performance, sensitivity and specificity characteristics, and further understanding is required. Our objective was to assess the accuracy of urinary antigen detection, direct fluorescent antibody (DFA) staining, serological testing and the polymerase chain reaction (PCR) method versus culture analysis (the reference standard) in patients suspected of being infected with Legionella or patients with laboratory-confirmed LD. We performed a MEDLINE search in November 2014. Two authors independently assessed the trials and extracted data. Pooled analysis was performed through Meta-DiSc version 1.4.

Result. The inclusion criteria were met by 11 studies. All the studies evaluated PCR and DFA tests to detect Legionella in clinical specimens, comparing them to culture techniques, and were included in the meta-analysis. The pooled sensitivity and specificity for PCR were 83 % [95 % confidence interval (CI): 79–87 %] and 90 % (95 % CI: 88–92 %), respectively. DFA was evaluated in one study and the sensitivity and specificity of this test were 67 % (95 % CI: 30–93 %) and 100 % (95 % CI: 91–100 %), respectively. PCR had high sensitivity and specificity for early diagnosis of LD.

Conclusion. Culture analysis is deemed necessary for epidemiological studies, molecular strain typing and antibiotic sensibility evaluations; however, the performance of PCR in recent studies calls for additional, well-designed studies in order to achieve the best standard test, which will enable optimization of the Legionella infection diagnostic.

INTRODUCTION

Legionellosis is an infection caused by Legionella spp. The diagnosis of high-risk patients should rely on microbiological tests which allow the etiology of this infection to be established [1, 2]. These tests should be specifically requested, as they are not routinely performed in the laboratory [3]. All cases have to be confirmed through the available methodology, which includes classical culture techniques (the reference standard), urinary antigen detection, direct fluorescent antibody assay (DFA), serological assay and nucleic acid amplification [4]. A confirmed case requires one of the following criteria: isolation of Legionella spp. in a clinical sample, antibody titre increase (4x) with the second titre being not less than 128 for Legionella pneumophila serogroup 1, or urinary positive antigen for L. pneumophila serogroup 1 [4]. These techniques have different performance, sensitivity and specificity characteristics, and also different sources of error and different limitations, and they require careful interpretation [2]. It is extremely important to obtain rapid and effective diagnosis of Legionnaires’ disease (LD) cases in order to provide timely and appropriate therapy, which decreases the morbidity and mortality rates and reduces the costs associated with this disease. Culture analysis is the reference standard for LD, and although it is essential in clinical and epidemiological research, it is time consuming and has low sensitivity, which does not allow for rapid diagnosis in severe cases of illness. Therefore, a systematic review of the literature for new methods is required in order to estimate the accuracy of the relevant diagnostic tests.

Our objective was to assess the accuracy of urinary antigen detection, direct fluorescent antibody (DFA) staining,
serological testing and the polymerase chain reaction (PCR) method versus culture analysis (the reference standard) in patients suspected of suffering from LD or patients with laboratory-confirmed LD.

METHODS

Search strategy and eligibility criteria

An electronic database search of MEDLINE (PubMed) was performed in November 2014. The reference lists of all the primary studies identified by the initial search were comprehensively evaluated to identify potential data. The search strategy is detailed in the Supplementary Data (online).

The literature search was restricted to studies published in English, Portuguese, French, Italian or Spanish.

Studies were considered eligible if they enrolled patients with confirmed LD (through culture analysis, urinary antigen detection or serological testing) or those with a high suspicion of Legionella infection. Studies had to evaluate in the same samples urinary antigen detection, serological testing, DFA staining and PCR compared to the culture method, which is the reference standard for LD diagnosis [2]. Studies needed to report sensitivity and specificity, or to report detailed data to derive true-positive, false-positive, false-negative and true-negative values for Legionella spp. Those that did not report the results of culture methods were excluded.

Study selection and data extraction

The references identified in the electronic search were independently screened by two authors (EC and DA) who examined the title and abstract for full-text assessment eligibility.

Study characteristics and results were extracted independently in a standardized form. Data were extracted from each individual study to retrieve the year of publication, study design, clinical setting, population, outcomes and methodological quality. The numbers of true positives, true negatives, false positives and false negatives were also recorded.

Methodological quality

The methodological quality of the included primary studies was assessed using a modified Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool [5], structured so that four key domains were each rated in terms of the risk of bias and concerns regarding applicability to the research question. The domains were patient selection, index test(s), the reference standard, flow and timing. Each key domain had a set of signalling questions to help reach judgements regarding bias and applicability. The extracted data were used to estimate sensitivity and specificity and investigate the diagnostic performance of each index test.

Investigations of heterogeneity

Factors that could influence diagnostic accuracy, other than true test performance, included those relating to the methodological quality and study design, the characteristics of the underlying population, and the characteristics of the index and reference test. We detailed and compared patient inclusion criteria for each included study. Factors such as differences in study population characteristics and test application (criteria for positive tests) were used to explore any heterogeneity and its impact on the relative accuracy. All the studies had similar characteristics and therefore there was no heterogeneity.

Statistical analysis

We extracted or derived data for the diagnostic performance presented in each primary study for each index test. Data were analysed using statistical software, Meta-DiSc version 1.4. We constructed 2×2 tables of true-positive cases, false-positive cases, false-negative cases and true-negative cases. We considered patients with culture-positive results as true positives and patients with culture-negative results as true negatives when analysing the performance of each index test. We calculated the sensitivity and specificity with 95 % confidence intervals (CI) in each study and created forest plots and graphs of the summary receiver operating characteristic (SROC) of the sensitivity and specificity for each index test to investigate diagnostic performance and heterogeneity when there were two or more estimates. Where there were studies of similar comparisons reporting the same outcome measures, a meta-analysis was undertaken.

RESULTS

The results for the electronic database searches and hand searching are outlined in Fig. S1 (available in the online Supplementary Material).

Included studies

We identified 11 studies that evaluated PCR and DFA tests [6–15]. A total of 2119 clinical samples were from patients who were suspected of having pneumonia caused by Legionella spp. All the studies evaluated PCR and DFA tests for the detection of Legionella in clinical specimens compared to culture techniques.

Nine studies evaluated PCR tests [6–9, 11–14], and two studies examined both PCR and DFA [10, 15] in the same patients and compared them against the gold standard.

The main characteristics of the included studies are summarized in Tables S1 and S2, together with details concerning the exclusion of some key studies.

Methodological quality of included studies

A methodological quality assessment was completed for each included study.

We identified 11 studies that compared PCR and DFA directly versus culture analysis for the early detection of LD. The studies were rated overall as having a low risk of bias and few concerns regarding applicability, with the exception of the index test risk of bias. In this case it was unclear whether the interpretation of the results of the index test was made without the knowledge of the reference standard.
These results are presented graphically in Figs S2 and S3.

**Pooled analyses**

PCR showed a pooled sensitivity of 83 % (95 % CI: 79–87 %) (Fig. 1), and a specificity of 90 % (95 % CI: 88–92 %) (Fig. 2). Only the studies by Edelstein and Wilson did not have a sensitivity of 100 % (Fig. 1). The AUC determined by the SROC curve was 0.98 (Fig. 3).

DFA was evaluated in one trial [15], and its sensitivity and specificity were 67 % (95 % CI 30–93 %) and 100 % (95 % CI: 91–100 %), respectively.

**DISCUSSION**

The main result of this review was the observation that PCR provides high sensitivity and specificity faster than the reference standard (culture analysis). Culture analysis has low sensitivity, of approximately 60 %, which is related to the fastidious nature of the bacterium, which requires 3–5 days to form visible colonies and must be examined by an experienced professional [2, 4]. The fact that the majority of patients have already started antibiotic therapy prior to sample collection is also associated with the low sensitivity of the reference standard. All these problems cause a delayed laboratory response, which does not meet a serious condition’s demand for urgent diagnosis. Despite the above characteristics, culture analysis has 100 % specificity for LD diagnosis.

Our results may provide new insights into *Legionella* diagnosis, as PCR may overcome some of the limitations of the reference standard and provide high sensitivity and specificity. PCR has the ability to detect DNA from damaged or dead bacteria rather than the viable bacteria needed for
detection by culture analysis, enabling the detection of all species of *Legionella*, and it has epidemiological utility for the investigation and management of outbreaks of LD. Only a few studies did not report a sensitivity of 100% for PCR. For example, the Edelstein study showed a lower performance of the Gene Probe kit due to the retrospective nature of this study and the nature of the specimen handling and storage. Precise handling procedures for samples were not recorded at the time of original freezing, and for this reason the quality of samples might have reduced the sensitivity of this test. According to the author, the results obtained can, at best, define the minimum performance of DNA assay.

The sensitivity and specificity obtained for DFA in our review (67 and 100%, respectively) show that its use in the clinical practice routine is limited and questionable as a fast first line of diagnosis.

**Limitations**

This was a systematic review with meta-analysis of relatively heterogeneous data (samples of different origins and PCR tests with different targets), which may have limited the conclusions. Furthermore, our data are limited by the methodological quality of the included studies.

**Conclusions**

PCR shows high sensitivity and specificity in the diagnosis of infections caused by *Legionella* spp. PCR seems to have the requirements needed for the quick and efficient diagnosis of *Legionella* infections. However, standardization is required for biological samples.

Additional, well-designed studies are needed in order to achieve the best standard test, which will enable optimization of the diagnosis of *Legionella* infection. These studies should include clinical and epidemiological data from patients, and samples subjected to the same collection conditions, transport, storage and management. PCR test should include targets against different regions of the bacterial genome in order to allow all species of *Legionella* spp. to be identified. The results of such experiments would be very helpful to clinicians and microbiologists, who are currently faced with a myriad of different tests, and this might help to establish standard methods that can not only be used in research, but also in daily clinical practice.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

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