Journal of Infection (2015) xx, 1–10

ELSEVIER

BIAM British Infection Association

www.elsevierhealth.com/journals/jinf

Molecular diagnosis of *Legionella* infections – Clinical utility of front-line screening as part of a pneumonia diagnostic algorithm

Naomi J. Gadsby [a,*], Kristjan O. Helgason [a,c], Elizabeth M. Dickson [a,d], Jonathan M. Mills [a,e], Diane S.J. Lindsay [b], Giles F. Edwards [b], Mary F. Hanson [a], Kate E. Templeton [a], in collaboration with the ESCMID Study Group for Molecular Diagnostics and the ESCMID Study Group for *Legionella* Infections, Basel, Switzerland

[a] *Medical Microbiology, Dept Laboratory Medicine, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh, EH16 4SA, UK*
[b] *Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory, New Lister Building, Glasgow Royal Infirmary, 10-16 Alexandra Parade, Glasgow, G31 2ER, UK*

Accepted 20 October 2015
Available online ■ ■ ■

KEYWORDS
Legionella pneumophila;
Legionnaires' Disease;
Pneumonia;
PCR;
Molecular diagnostics;
Legionella longbeachae;
Legionella anisa;
Legionella hackeliae;
Legionella species

Summary *Objectives:* Urinary antigen testing for *Legionella pneumophila* serogroup 1 is the leading rapid diagnostic test for Legionnaires' Disease (LD); however other *Legionella* species and serogroups can also cause LD. The aim was to determine the utility of front-line *L. pneumophila* and *Legionella* species PCR in a severe respiratory infection algorithm.
Methods: L. pneumophila and *Legionella* species duplex real-time PCR was carried out on 1944 specimens from hospitalised patients over a 4 year period in Edinburgh, UK.
Results: L. pneumophila was detected by PCR in 49 (2.7%) specimens from 36 patients. During a LD outbreak, combined *L. pneumophila* respiratory PCR and urinary antigen testing had optimal sensitivity and specificity (92.6% and 98.3% respectively) for the detection of confirmed cases. *Legionella* species was detected by PCR in 16 (0.9%) specimens from 10 patients. The 5 confirmed and 1 probable cases of *Legionella longbeachae* LD were both PCR and antibody positive.

* Corresponding author. Tel.: +44 (0)131 242 6069; fax: +44 (0)131 242 6008.
E-mail address: naomi.gadsby@luht.scot.nhs.uk (N.J. Gadsby).
[c] Present address: Department of Microbiology, Landspitali, University Hospital of Iceland, 101 Reykjavik, Iceland.
[d] Present address: Scottish MRSA Reference Laboratory, New Lister Building, Glasgow Royal Infirmary, 10-16 Alexandra Parade, Glasgow, G31 2ER, UK.
[e] Present address: Department of Medical Microbiology, Raigmore Hospital, Old Perth Road, Inverness, IV2 3UJ, UK.

http://dx.doi.org/10.1016/j.jinf.2015.10.011

Please cite this article in press as: Gadsby NJ, et al., Molecular diagnosis of *Legionella* infections – Clinical utility of front-line screening as part of a pneumonia diagnostic algorithm, J Infect (2015), http://dx.doi.org/10.1016/j.jinf.2015.10.011

Conclusions: Front-line *L. pneumophila and Legionella* species PCR is a valuable addition to urinary antigen testing as part of a well-defined algorithm. Cases of LD due to *L. longbeachae* might be considered laboratory-confirmed when there is a positive *Legionella* species PCR result and detection of *L. longbeachae* specific antibody response.

Introduction

Legionella is a significant cause of community and hospital-acquired pneumonia, with sporadic cases and outbreaks of Legionnaires' Disease (LD) reported worldwide. *Legionella pneumophila* serogroup 1 (sg1) is the major cause, but it can also be caused by other serogroups and by *Legionella* species other than *L. pneumophila* (*Legionella* species). The commonly used urinary antigen test is convenient because patients with LD may be unable to produce a respiratory specimen, but it requires a specific request by the clinician. Furthermore, currently available urinary antigen tests are designed to detect *L. pneumophila* sg1 primarily, thus reducing their sensitivity for overall LD diagnosis.[1] As recently reviewed, there is now a diagnostic gap in routine testing for LD following the widespread use of urinary antigen testing and subsequently, *L. pneumophila* sg1 may be overestimated in current estimates of LD.[2]

Legionella species other than *L. pneumophila* are increasingly recognised as causes of severe lower respiratory tract infection and *Legionella longbeachae*, in particular, is an important etiological agent of community-acquired pneumonia in Australia and New Zealand.[3–6] *L. longbeachae* sg1 infections have also been recently detected in the UK in association with the use of potting compost.[7,8] The latest data from Europe suggest that 15% of LD cases are due to *Legionella* species other than *L. pneumophila* sg1; however few front-line diagnostic laboratories routinely screen for these organisms.[9] Multiplex real-time PCR for the rapid detection of *L. pneumophila* and *Legionella* species has been well described, but is not widely deployed.[6,10–15] Therefore, it is likely that cases of respiratory illness due to *Legionella* species, including non-sg1 *L. pneumophila,* are occurring but not identified.

Duplex real-time PCR for *L. pneumophila* and *Legionella* species has been used in the Royal Infirmary of Edinburgh Microbiology Laboratory as part of a front-line syndromic molecular testing algorithm for severe respiratory tract infection, since March 2010. This algorithm also includes simultaneous testing for nine respiratory viruses along with the atypical bacterium *Mycoplasma pneumoniae*, and is complemented by urinary antigen testing for *L. pneumophila* and routine microbiological culture. The aim of this study was to determine the utility of the *L. pneumophila* and *Legionella* species PCR testing algorithm in patients with severe pneumonia over a four-year period of routine use, during which a large outbreak of LD occurred in Edinburgh.[16,17]

Materials and methods

Clinical cases and specimens

Laboratory records were obtained for all specimens tested by duplex real-time PCR for *L. pneumophila* and *Legionella* species over a 44 month period between 1st March 2010 and 31st October 2013 in the Royal Infirmary of Edinburgh Microbiology Laboratory. Results were available for both *L. pneumophila* and *Legionella* species for 1736 specimens, *L. pneumophila* only for 181 specimens and *Legionella* species only for 27 specimens. EU case definitions were used for LD in patients with a clinical diagnosis of pneumonia[18] (Table 1). The study was carried out as part of a clinical audit of routinely gathered clinical data for a local clinical governance programme and was approved by the Quality Improvement Team, Royal Infirmary of Edinburgh. Handling and testing of anonymised specimens was carried out in accordance with ethical approval from the Lothian Regional Ethics Committee (08/S11/02/2).

Legionella testing algorithm

L. pneumophila and *Legionella* species PCR was carried out routinely on a defined subset of respiratory specimens received by the laboratory, whether or not *Legionella* testing had been specifically requested, according to a defined algorithm (Fig. 1). Specimen testing occurred 7 days a week. The algorithm was modified in response to a LD outbreak that occurred in Edinburgh between 28th May and 13th July 2012 to include the routine testing of lower respiratory tract (LRT) specimens received from all respiratory wards in hospitals in the NHS Lothian health board. Specimens were also accepted from local community hospitals and primary care clinics for patients meeting the outbreak epidemiological case definition. For the latter group, respiratory viral PCR was carried out retrospectively due to workload pressures at the time of the outbreak. Clinicians treating suspected LD cases were advised to submit all of the following specimens where possible: (1) urine for immunochromatographic (IC) urinary antigen testing for *L. pneumophila* sg1; (2) LRT specimen for microbiological culture and syndromic respiratory molecular testing including *Legionella* PCR; (3) paired acute and convalescent sera for *Legionella* serological testing.

Syndromic respiratory molecular testing protocol

Total nucleic acid was extracted from specimens accepted for *Legionella* PCR testing using the automated nucliSENS easyMAG (bioMérieux, Basingstoke, UK) system with off-board lysis, specified *Legionella* DNA-free reagents (obtained through discussion with the manufacturer), input volume of 200 μl and elution volume of 100 μl for all specimen types. The same extract was used in five multiplex PCR assays in a panel comprising: (1) real-time duplex fast PCR for the detection of *Legionella* species (16S rRNA gene) and *L. pneumophila* (mip gene) as previously described[11] with modification for hydrolysis probes [*Legionella* species probe sequence:

Please cite this article in press as: Gadsby NJ, et al., Molecular diagnosis of *Legionella* infections — Clinical utility of front-line screening as part of a pneumonia diagnostic algorithm, J Infect (2015), http://dx.doi.org/10.1016/j.jinf.2015.10.011