Routine PCR testing for *Legionella* species as part of a front-line syndromic molecular testing algorithm for pneumonia patients in the UK

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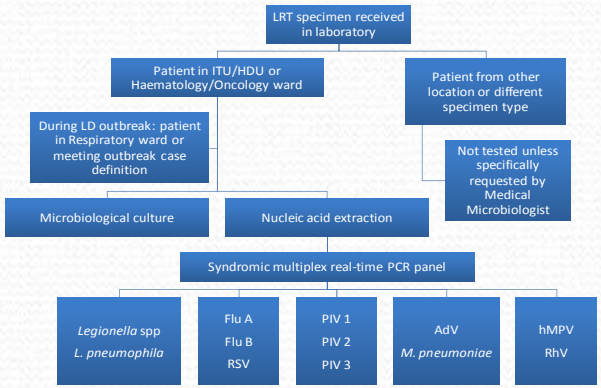
Introduction and Purpose

- Front-line diagnostic testing for Legionnaires' Disease (LD) relies predominantly on urinary antigen testing. This test is rapid and convenient and does not require patients with LD to produce a respiratory specimen. However, currently available urinary antigen tests are designed to detect only *Legionella pneumophila* serogroup 1
- Although *L. pneumophila* sg1 is the leading cause of LD in Europe, 15% of LD cases can be caused by other *Legionella* species and other *L. pneumophila* serogroups [1]. In particular, several *L. longbeachae* sg1 infections have been recently detected in the UK in association with the use of potting compost [2]
- *L. pneumophila* and other *Legionella* species can be rapidly detected in respiratory specimens using real-time PCR but few front-line diagnostic laboratories routinely use molecular assays for *Legionella* detection
- The aim of this study was to determine the utility of routine *L. pneumophila* and *Legionella* species PCR testing of respiratory tract samples as part of a syndromic molecular testing algorithm for respiratory pathogens in Edinburgh, UK

[1] Beauté J, Zucs P, de Jong B. Euro Surveill 2013; 18(10): 20417
[2] Lindsay DS, Brown AW, Brown DJ et al. J Med Microbiol 2012; 61(Pt 2): 218-222

Methods

- **Use of defined testing algorithm**
- **Study period:** 1st March 2010 to 31st October 2013 (44 months)
- **Location:** Microbiology laboratory of tertiary care hospital in Edinburgh, UK
- **Respiratory specimens:** 1944 specimens from patients as per testing algorithm
- **Nucleic acid extraction:** automated nucliSENS easyMAG (bioMérieux) system with off-board lysis and specified *Legionella* DNA-free reagents
- ***Legionella* molecular testing:** real-time duplex fast PCR for the detection of *Legionella* species (16S rRNA gene) and *L. pneumophila* (mip gene) [3]
- **Additional *Legionella* testing:**
  - BinaxNOW *Legionella* Urinary Antigen test (Alere) as per testing algorithm.
  - Specific culture for *Legionella* was attempted for specimens from patients with a positive urinary antigen or PCR test.
  - Referral to reference laboratory of specimens positive by any method



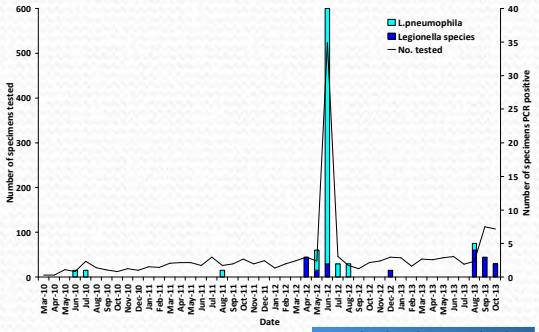
EU case definitions for Legionnaires' Disease in patients with a clinical diagnosis of pneumonia

Case	Laboratory Criteria
Confirmed	At least one of the following: 1. Isolation of *Legionella* species from respiratory secretions or any normally sterile site 2. Detection of *L. pneumophila* antigen in urine 3. Detection of *L. pneumophila* sg1 specific antibody response
Probable	At least one of the following: 1. Detection of *Legionella* species nucleic acid in a clinical specimen 2. Detection of *L. pneumophila* non-sg1 or other *Legionella* species specific antibody response 3. Detection of a single high titre in specific serum antibody for *L. pneumophila* sg1, other serogroups or other *Legionella* species

[3] Templeton KE, Scheltinga SA, Sillekens P et al. J Clin Microbiol 2003; 41(9): 4016-21

Results

- *L. pneumophila* was detected by PCR in 49 (2.7%) specimens from 36 patients
  - 28 were confirmed cases of LD
  - 8 were probable cases of LD
- There was a large outbreak of LD in south west Edinburgh between 28/05/12 and 13/07/12 due to *L. pneumophila* sg1 (Knoxville) sequence type 191
- During the LD outbreak, the combination of *L. pneumophila* PCR and/or urinary antigen testing gave optimal sensitivity and specificity for the detection of confirmed cases



PCR and Ag testing during LD outbreak		Confirmed case		Sensitivity	Specificity
		Yes*	No		
L. pneumophila PCR	+	25	5#	83.3%	99.0%
	-	5	486		
Urinary antigen test	+	41	0	87.2%	100.0%
	-	6	1402		
L. pneumophila PCR+/- urinary antigen test	+	46	5#	95.8%	99.7%
	-	2	1492		

* 48 patients tested locally were confirmed cases according to EU laboratory criteria
# defined as probable cases

- The inclusion of molecular testing for respiratory viruses identified patients hospitalised with viral pneumonia who were clinically indistinguishable from those with LD

Results of respiratory syndromic molecular testing during LD outbreak	
Organism	No. positives
Influenza A	4
Influenza B	4
RSV	0
PIV1	1
PIV2	0
PIV3	21
Adenovirus	11
hMPV	12
Rhinovirus	81
M. pneumoniae	7

- *Legionella* species were detected by PCR in 16 (0.9%) specimens from 10 patients
- 7 required critical care admission with pneumonia
- Epidemiological investigation demonstrated that many patients had significant exposure to potting compost before their illness

Patient	Date of Diagnosis	*Legionella spp* PCR result	*Legionella* culture result	*Legionella* serological test result	Diagnosis according to EU case definition
1	Apr 2012	Positive	No growth	>four-fold rise to *L. hackeliae* sg 2	Probable *L. hackeliae*
2	Apr 2012	Positive	No growth	>fourfold rise to *L. anisa*	Probable *L. anisa*
3	May 2012	Positive	No growth	>fourfold rise to *L. longbeachae* sg 1	Probable *L. longbeachae*
4	Jun 2012	Positive	No growth	Not performed	Probable *Legionella* species
5	Dec 2012	Positive	No growth	Not performed	Probable *Legionella* species
6	Aug 2013	Positive	*L. longbeachae* sg 1	Rise to *L. longbeachae* sg 1 From titre of 64 to 256	Confirmed *L. longbeachae*
7	Aug 2013	Positive	*L. longbeachae* sg 1	>four-fold rise to *L. longbeachae* sg 1	Confirmed *L. longbeachae*
8	Aug 2013	Positive	*L. longbeachae* sg 1	>four-fold rise to *L. longbeachae* sg 1	Confirmed *L. longbeachae*
9	Sep 2013	Positive	*L. longbeachae* sg 1	Rise to *L. longbeachae* sg 1 From titre of 256 to 512	Confirmed *L. longbeachae*
10	Oct 2013	Positive	*L. longbeachae* sg 1	Not performed	Confirmed *L. longbeachae*

Conclusions

- A syndromic respiratory molecular testing algorithm including *L. pneumophila* and *Legionella* species PCR enabled early detection and rapid response to a large outbreak of LD in Edinburgh in 2012
- We also identified several cases of severe pneumonia due to *Legionella* species, particularly *L. longbeachae* in 2013, which would have gone undetected by relying on urinary antigen testing alone
- We propose that a positive *Legionella* species PCR result plus a specific antibody response to *L. longbeachae* is sufficient evidence to consider *L. longbeachae* infection as a laboratory-confirmed case of LD

Acknowledgments

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