Impact of UAT Diagnostic Methods on Estimates of Legionnaires' disease Caused by non-*pneumophila Legionella*

Supplementary Information for:
Legionnaires' Disease Surveillance and Public Health Policies in Italy: A Mathematical Model for Assessing Prevention Strategies

Although the total number of Legionnaires' disease cases is certainly underestimated in all regions when diagnoses are based primarily on urine antigen test (UAT) results, the ratio of total Legionnaires' disease cases (including both diagnosed and non-diagnosed) caused by all serogroups of *L. pneumophila* is very unlikely to have been biased by the recent decades of use of urine antigen testing.

As described below, data from both Europe and the U.S. demonstrate that UAT-only testing regimens do not lead to underestimations of the percentage of Legionnaires' disease cases caused by non-*pneumophila* species. Instead, UAT diagnostic regimes are likely to undercount far more cases caused by *L. pneumophila* SG2-15 than to undercount cases caused by other, non-*pneumophila* species.

- Ten years of ECDC surveillance data demonstrated that whether UAT was employed as the primary diagnosis tool or not, the proportion of Legionnaires' disease cases (95%+) caused by *L. pneumophila* (SG1 and SG 2-15) did not change.
- In New York, the etiology of Legionnaire's disease cases did not change materially after the widespread adoption of UATs.
- In France and Denmark, Legionnaires' disease cases diagnosed without any reliance on UAT still show 97% and 93% respectively of cases are caused by one of the *L. pneumophila* serogroups.
- These findings align with data from multiple countries showing that the *Legionella* species most commonly found in environmental samples do not correlate with the almost exclusively *L. pneumophila* isolates from patients with Legionnaires' disease.

Total Legionnaires' disease prevalence is underestimated when based solely on urine antigen test results.

It is often asserted that the use of a UAT as the primary tool for diagnosing patients with Legionnaires' disease leads to underestimation of the true number of cases because UATs detect only the presence of the *L. pneumophila* serogroup 1 antigen in clinical samples, rather than detecting antigens of all potential *Legionella* species and serogroups which have been documented

to cause the disease. This is certainly true. Both groups of patients infected with a) any of the *L. pneumophila* serogroups 2-15, as well as b) patients with illness caused by non-*pneumophila* species, are not identified as Legionnaires' disease cases unless they are diagnosed by an alternative or supplementary method such as a qPCR or culture test.

Unfortunately, one of the early and commonly cited studies (Benin et al) describing the under-diagnosis of overall Legionnaires' disease not attributed to *L. pneumophila* SG1 after the widespread adoption of UAT testing did not differentiate between the cases missed that were due to other serogroups of *L. pneumophila* (SG2-15) and cases missed due to non-*pneumophila.* This may have led readers to incorrectly attribute the undercounting of cases exclusively to non-*pneumophila* species.[1,2]

Use of urine antigen tests for primary diagnoses does not materially change the percentage of total Legionnaires' disease cases which are caused by non-pneumophila Legionella species.

Even though the <u>overall</u> Legionnaires' disease case burden is underestimated when only cases initially diagnosed with UAT are counted, a close examination of the data shows that the <u>percentage</u> of total Legionnaires' disease cases caused by non-*pneumophila* species remains relatively consistent whether or not UAT is used as the initial diagnostic method. The *L. pneumophila* serogroup 1 specificity of UATs means that cases caused not only by non-*pneumophila* species but also by all other serogroups of *L. pneumophila* (SG2-15) go undiagnosed.

In fact, research has shown that UAT-only testing regimens are likely to undercount far more Legionnaires' disease cases caused by *L. pneumophila SG2-15* than they undercount Legionnaires' disease cases caused by other non-*pneumophila Legionella* species.[3] Even though both are missed by UAT, the proportion of non-*pneumophila* etiology cases relative to the total cases is not significantly influenced by this initial UAT diagnosis bias. As described below, this conclusion is supported by a 10-year pan-European ECDC dataset analyzed by Beauté et al, a recent analysis of over 23,000 clinical test results in the U.S. specifically focusing on potential UAT bias, and the results of several years of etiology reporting by the Statens Serum Institute in Denmark.

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[1] Benin, Andrea L et al. "Trends in legionnaires disease, 1980-1998: declining mortality and new patterns of diagnosis." *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* vol. 35,9 (2002): 1039-46. doi:10.1086/342903

[2] Benin et al found a 79% drop in Legionnaires' disease cases not attributed to Lp SG1 (from 28% to 4% of total cases) during the fifteen-year period from 1983 to 1998 which saw the switch to widespread adoption of UAT testing and this statistic is often cited. (Benin, 2002).[2] Unfortunately, this paper did not report what portion of the 28% rate of *non-Lp* SG 1 to total positive specimens documented in 1983 was made up of *non-pneumophila Legionella* and what portion was made up of *L. pneumophila* SG 2-15. Nor did the paper include what portion of this 79% reduction in the percent of *Non-Lp* SG1 to total cases was due to a reduction in non-Lp specimens and what portion was due to fewer SG2-15 Lp specimens.

[3] Schoonmaker-Bopp D, Nazarian E, Dziewulski D, Clement E, Baker DJ, Dickinson MC, Saylors A, Codru N, Thompson L, Lapierre P, Dumas N, Limberger R, Musser KA. 2021. Improvements to the success of outbreak investigations of Legionnaires' disease: 40 years of testing and investigation in New York State. Appl Environ Microbiol 87: e00580-21. https:// doi.org/10.1128/AEM.00580-21

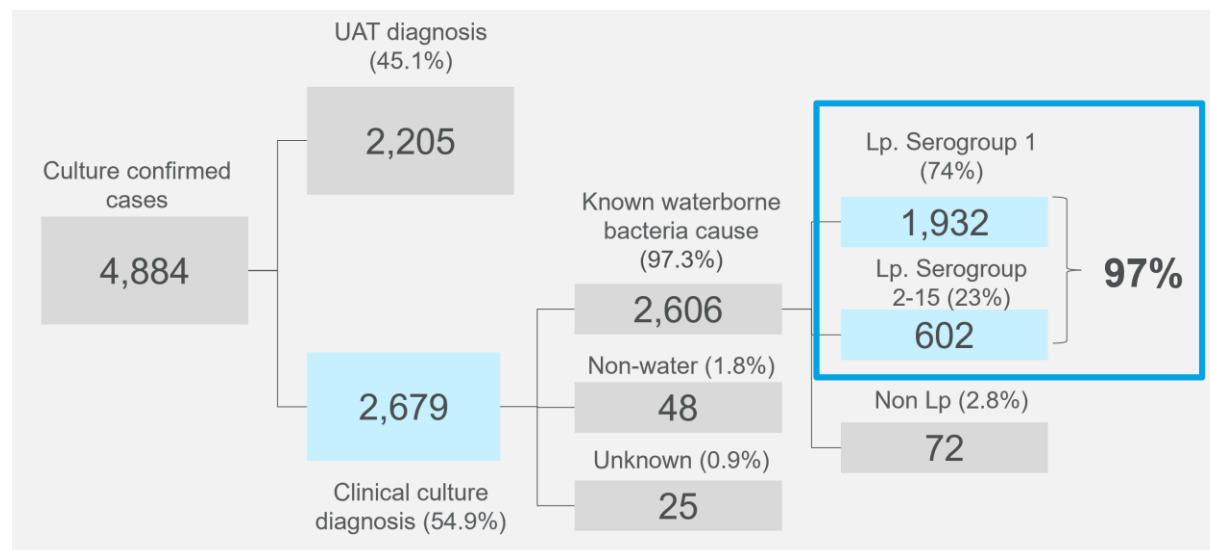*Etiological analysis of European clinical data: 2008-2017*

Beauté et al compared community-acquired Legionnaires' disease cases with healthcare-associated (HCA) Legionnaires' disease cases using ten years (2008-2017) of data from the European Surveillance System (TESSy).[4] The study covered 29 EU countries, reporting 40,411 community-acquired and 4315 HCA cases. The study included all locally acquired cases that met the 2012 EU-EEA case definition of confirmed and probable cases of Legionnaires' disease.

4884 of the Legionnaires' disease cases reported were culture-confirmed and 55% of those cases did <u>not</u> have a prior UAT diagnosis. Culture confirmation was more common for HCA Legionnaires' disease cases than community-acquired cases (15.9% vs 10.4%).

Further analysis of the Beaute data reveals the following:

- For the 2679 culture-confirmed cases initially diagnosed <u>without</u> the use of a UAT, 95% of total cases with a known cause, and 97% of the 2606 cases associated with waterborne bacteria (e.g. excluding *L. longbeachae*) were caused by *L. pneumophila (SG1 & SG2-15)*

Figure 1: Etiology of culture-confirmed Legionnaires' disease cases initially diagnosed without the use of UAT (European Clinical Data 2008-2017)

[4] Beauté, Julien et al. "Healthcare-Associated Legionnaires' disease, Europe, 2008- 2017." Emerging infectious diseases vol. 26,10 (2020): 2309-2318. doi:10.3201/eid2610.181889

- 97% of total culture-confirmed cases (with and without prior UAT diagnoses) were caused by *L. pneumophila*. Proportions were similar for community-acquired and healthcare-associated (HCA) Legionnaires' disease cases (97.4% vs 98.1%)

Figure 2: Etiology of all culture-confirmed Legionnaires' disease cases, both community-acquired and healthcare-associated cases (European Clinical Data 2008-2017)
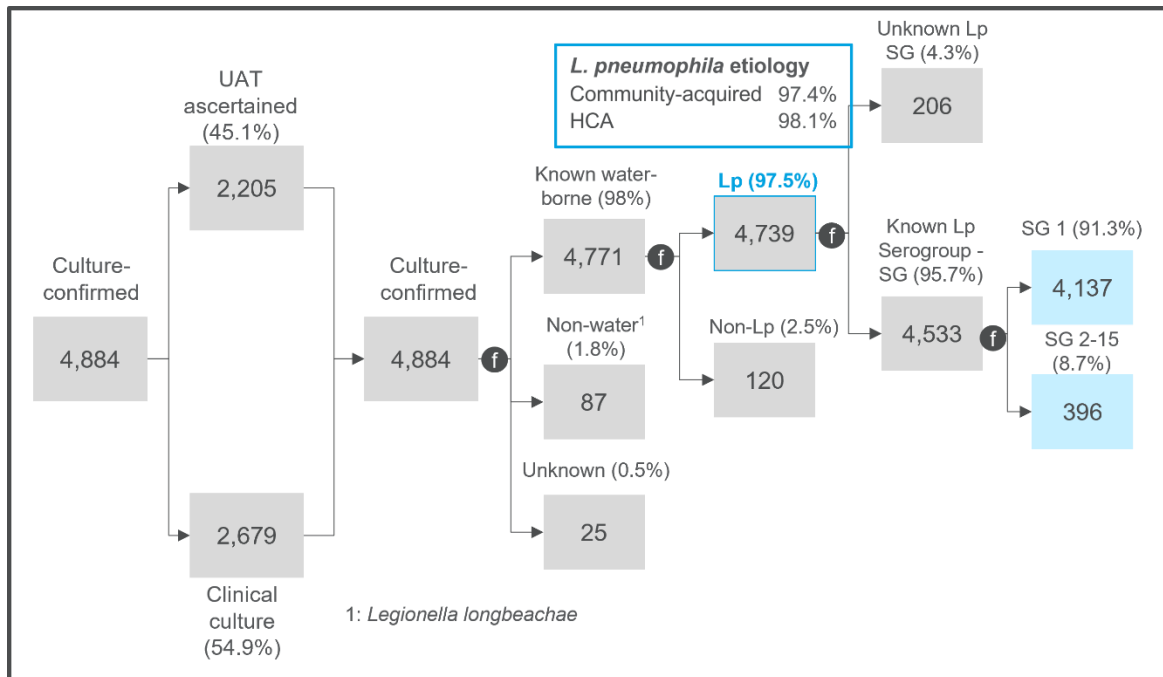
In summary, ten years of ECDC surveillance data demonstrated that, whether or not UAT was employed as the primary diagnosis tool did not s change the proportion (95%+) of Legionnaires' disease cases that were reported to be caused by *L. pneumophila* (SG1 and SG2-15).

Legionnaires' disease case etiology based on U.S. Clinical Data: 1978-2017

Researchers from the New York State Department of Health reviewed 40 years of clinical samples from Legionnaires' disease patients received by the Department between 1978 and 2017, a database of more than 23,000 clinical test results (Schoonmaker-Bopp et al.)[5]. They identified 626 total positive clinical samples of which 70% were determined to be outbreak-associated and 30% were sporadic. The researchers compared the frequency of *L. pneumophila* SG1, *L. pneumophila*

[5] Schoonmaker-Bopp D, Nazarian E, Dziewulski D, Clement E, Baker DJ, Dickinson MC, Saylors A, Codru N, Thompson L, Lapierre P, Dumas N, Limberger R, Musser KA. 2021. Improvements to the success of outbreak investigations of Legionnaires' disease: 40 years of testing and investigation in New York State. Appl Environ Microbiol 87: e00580-21. Table 4 https:// doi.org/10.1128/AEM.00580-21.

non-SG1, and non-*pneumophila* species isolated from the total set of positive clinical specimens before (140 samples) and after (486 samples) the widespread use of UAT diagnoses in New York, defined as beginning in 2000.

The researchers found an 18% increase in *L. pneumophila* SG1 from the isolated clinical strains as a proportion of the total *Legionella* strains isolated over the period analyzed. This change encompassed both a decrease in the percentage of other *L. pneumophila* serogroup (SG2-13) strains isolated and a decrease in the number of non-*pneumophila* species strains isolated. The researchers found that for clinical samples taken <u>before</u> widespread UAT use (i.e., before 2000), the percentage of *L. pneumophila* non-SG1 strains was 2.4 times higher than the % of *L. pneumophila* non-SG1 strains found in the period after widespread UAT utilization (23.6% vs 9.7%). The percentage of non-*pneumophila* strains to total *Legionella* positive clinical specimens taken before widespread UAT use was only 1.5 times higher than during the primary UAT utilization period (10.7% vs. 7%).[6] (See Table 4 in Schoonmaker-Bopp and Table A below.)

The data from Schoonmaker-Bopp et al has an average "pre-UAT" rate of non-*pneumophila* *Legionella* of 10.7% of total specimens. The high percentage of non-*pneumophila* species cases relative to what is typically seen in the literature was heavily driven (8.2% out of 10.7%) by a single 1995-96 outbreak of *L. micdadei* in the transplant unit of a large tertiary-care hospital. Although the percentage of the positive clinical samples tied to healthcare-associated Legionnaires' disease cases is not reported in the study, 85% of the outbreak sources for which facilities were identified were healthcare facilities. The sporadic case investigations included 21 healthcare facilities and 25 other buildings. As such, the data set seems to reflect a predominately nosocomial, rather than community-acquired, Legionnaires' disease patient population.

TABLE A

All Legionella strains isolated from clinical specimens 1978-2017									Non Lp SG1 underestimation factor	
	Number Pre UAT	Number Post UAT			Pre UAT	Post UAT				
Lp SG1	92	405		Lp SG1	66%	83%				
Lp non-SG1	33	47		Lp non-SG1	24%	10%		**Lp non-SG1**	**2.44**	
Non Lp	15	34		Non Lp	11%	7%		**Non Lp**	**1.53**	
Total	140	486		Total	100%	100%				

[6]Schoonmaker-Bopp D, Nazarian E, Dziewulski D, Clement E, Baker DJ, Dickinson MC, Saylors A, Codru N, Thompson L, Lapierre P, Dumas N, Limberger R, Musser KA. 2021. Improvements to the success of outbreak investigations of Legionnaires' disease: 40 years of testing and investigation in New York State. Appl Environ Microbiol 87: e00580-21. Table 4 https:// doi.org/10.1128/AEM.00580-21.

Implications of NY State Experience for European Data

The 2.4 times under-estimation factor for *L. pneumophila* non-SG1 and the 1.5 times underestimation factor for non-*pneumophila* species found in NY can be applied to other data sets. For example, applying these adjustments to the 2008 to 2017 ECDC data set for all culture-confirmed cases analyzed by Beaute et al. generates an estimate of 3.7% non-*pneumophila* cases, only slightly higher than the 2.5% rate found among samples from patients who were not diagnosed based on UAT results (Table B).

TABLE B

Implications of NY State Experience for European Data

Total Culture Confirmed Cases including with UAT Initial Diagnosis - Community Acquired	Total Culture Confirmed Cases including with UAT Initial Diagnosis - Community Acquired	Total Culture Confirmed Cases including with UAT Initial Diagnosis - Healthcare Associated	Total	%	Adjustment for potential UAT underestimation (NY State Data)
Lp. SG1	3600	537	4137	85.1%	
Lp. SG 2-15	471	131	602	12.4%	2.4
Total Lp. (SG1-15)	4071	668	4739	97.5%	
Non Lp.	107	13	120	2.5%	1.5
Total	4178	681	4859		

Applying the same NY state UAT underestimation statistics to the most recent single year of ECDC data, from 2021, for which 89% of cases were diagnosed with UAT, the total share of cases attributed to non-*pneumophila* species from a mix of nosocomial and community settings would be 5% (Table C).[7]

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[7] European Centre for Disease Prevention and Control. Legionnaires' disease. In: ECDC. Annual Epidemiological Report for 2021. Stockholm: ECDC; 2023

TABLE C

2021 ECDC Culture Confirmed Samples (mix of nosocomial and community-acquired)							
	Number	%	Adjustment for initial UAT diagnosis (from NYS experience)	UAT Diagnostic Adjusted % of clinical samples			% when adjusted for potential UAT bias
Lp. SG1	890	87.5%		72%		Total Lp.	95%
Lp. non-SG1	95	9.3%	2.4	23%		Total non-*pneumophila*	5%
Non Lp.	32	3.1%	1.5	5%			
Total	1017			100%			

Danish PCR diagnosis clinical data

Several years of etiology reporting by the Statens Serum Institute in Denmark based on 93%+ initial PCR diagnoses in the population, e.g. when UAT is rarely employed, also show a low (7%) percentage of total Legionnaires' disease cases caused by non-*pneumophila Legionella* (with one non-*pneumophila* case ultimately confirmed in 2019, two in 2020, meaning confirmed cases from non-*pneumophila* were even lower).[8,9] The fact that non-*pneumophila* caused cases out of the total Legionnaires' disease cases documented when UAT underestimations were not a significant factor in the diagnoses were only 4% above the 2.7% proportion of non-*pneumophila* found in the wider European ECDC dataset (where UAT typically accounts for 88-90% of testing) reinforces the findings that UAT bias does not materially underestimate the proportion of Legionnaires' disease cases caused by other non-*pneumophila Legionella* species.

Disease case etiologies do not match the environmental distribution of Legionella *species*

Within the different studies, a most fundamental explanation for why reliance on UAT testing does not meaningfully change the portion of *L. pneumophila* and non-*pneumophila* cases is explored by Mercante et al.[10] He explains that the frequency of different *Legionella* species found in environmental samples has repeatedly been shown not to correlate with the species identified from clinical cases in the same regions which are instead heavily weighted toward *L. pneumophila* SG1. Across France, for example, when analyzing data from confirmed nosocomial cases, Doleans

[8] Statens Serum Institute (SSI) Legionnaires' disease in Denmark 2020. EPI-NEWS Nos 18 [Internet]. 2021 [cited 2022 Apr 6]; Available from: https://en.ssi.dk/news/epi-news/2021/ no-18---2021 15

[9] Statens Serum Institute (SSI) Legionella 2020 Annual Report. [internet] 2021 [cited April 6, 2022]. Available from https://en.ssi.dk/ surveillance-and-preparedness/ surveillance-in-denmark/annual-reportson-disease-incidence/legionella-2020- annual-report.

[10] Mercante JW, Winchell JM. 2015. Current and emerging Legionella diagnostics for laboratory and outbreak investigations. Clin Microbiol Rev 28:80 –118. doi:10.1128/CMR.00029-14.

et al found that although *L. pneumophila* SG1 accounted for only 27.4% of environmental isolates, 97.6% of clinical isolates were *L. pneumophila* SG1. The reverse was also true: isolates other than SG1 represented 72.6% of the environmental isolates but constituted only a total of 2.4% of the clinical isolates from Legionnaires' disease cases. Doleans et al. concluded that their findings reinforced the observation that non-*pneumophila* species are less pathogenic than *L. pneumophila*, which made them a much lower public health concern.[11]

*Healthy populations appear to develop antibodies but not Legionnaires' disease in response to non-*pneumophila *exposure.*

Various studies of different *Legionella* species antibodies within both healthy populations and ill patient groups point to the same conclusion. Healthy adults routinely exposed to non-*pneumophila* species develop antibodies but, because of lower virulence, do not become ill as result of this exposure. Lee et al. examined 500 healthy adults in one Korean province and found that 33%, 20%, and 15% of this population reacted to *L. bozemanii, L. micdadei,* and *L. longbeachae* antibody tests, respectively (along with 10% *L. pneumophila* SG 6). However, *L. pneumophila* SG1, SG2 or SG 3 antibodies were not found in any of these healthy individuals.[12] As early as 1987 in the U.S., Bornstein et al similarly found almost no *L. pneumophila* antibodies (from zero to 2.5% for the various serogroups) amongst 583 healthy blood donors whereas 15.5% of those healthy donors were found to have *L. bozemani* antibodies (the only non-*pneumophila* species antibody for which they were tested) and no evidence of past or present Legionnaires' disease was mentioned.[13]

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[11] Doleans A, Aurell H, Reyrolle M, Lina G, Freney J, Vandenesch F, Etienne J, Jarraud S. Clinical and environmental distributions of Legionella strains in France are different. J Clin Microbiol. 2004 Jan;42(1):458-60. doi: 10.1128/JCM.42.1.458-460.2004.

[12] Lee HK, Woo MK, Ju YI, Baek SJ, Song HJ, Choi JS, Kweon SS, Jeon DY, Kang YH. Prevalence of antibodies in response to Legionella species, analysis of a healthy population from Jeollanam-do Province, Korea. J Microbiol. 2008 Apr;46(2):160-4. doi: 10.1007/s12275-007-0181-9.

[13] Bornstein N, Janin N, Bourguignon G, Surgot M, Fleurette J. 1987. Prevalence of anti-Legionella antibodies in a healthy population and in patients with tuberculosis or pneumonia. Pathol Biol (Paris) 35:353–356

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Mercante JW, Winchell JM. 2015. Current and emerging Legionella diagnostics for laboratory and outbreak investigations. Clin Microbiol Rev 28:80 –118. doi:10.1128/CMR.00029-14.

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