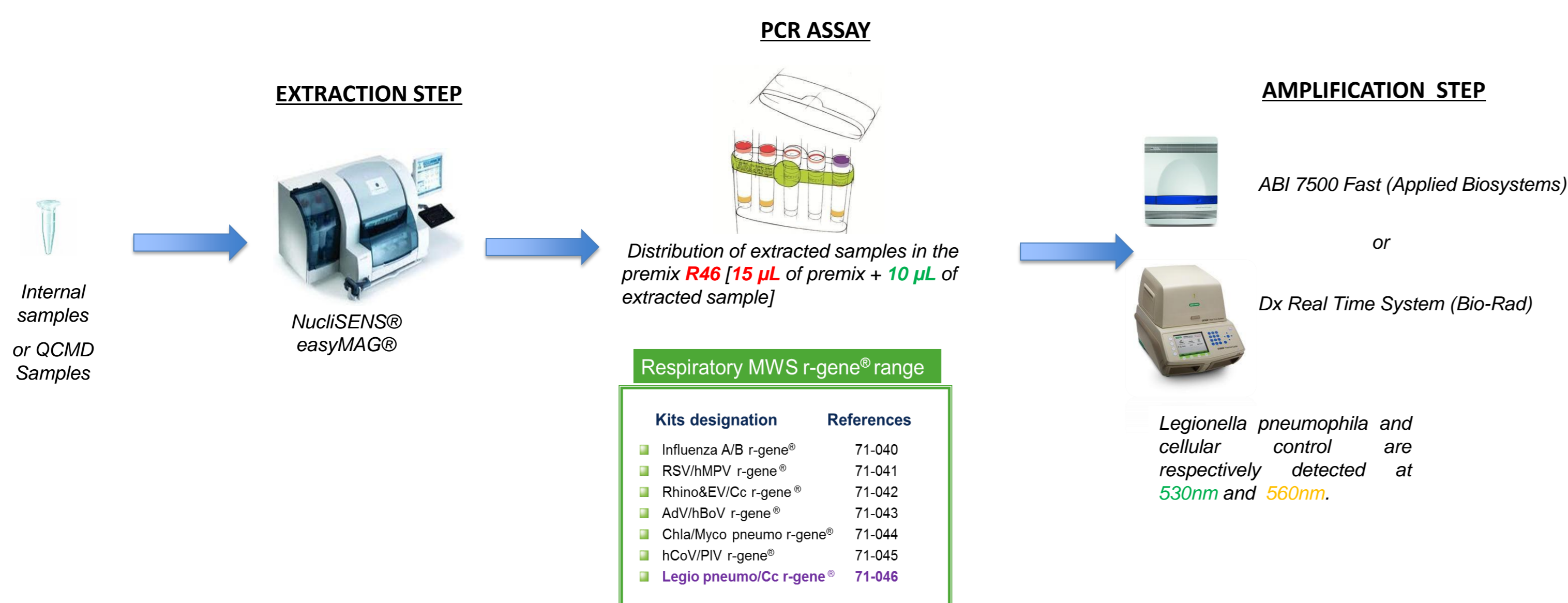


INTRODUCTION

Legionella pneumophila (Lp) is an intracellular and Gram negative bacterium primarily responsible for atypical pneumonia. Lp contains 15 serogroups (Sg). Its rapid detection is difficult with immunological tests or culture methods. Furthermore, antigen detection in urine is possible only for Sg 1. The new Respiratory MWS r-gene[®] real time PCR Legio pneumo/Cc r-gene[®] kit (bioMérieux, ref : 71-046) allows the qualitative detection of *Legionella pneumophila* Sg 1 to 15 in respiratory samples (bronchoalveolar lavage fluid, sputum, tracheobronchial aspirate fluid) using 5' nuclease technology. This new product allows a simplification for the etiological diagnostic of atypical pneumonia. Speed and sensitivity of the results contribute to a better management of patients. *Legionella pneumophila* detection is associated with a cellular control (Cc) to check the presence of cells in the sample and thus to validate the sampling. The results of the analytical sensitivity, the limit of detection, the reactivity, the precision and a QCMD panel testing are presented.

MATERIAL AND METHODS

- ✓ **AMPLIFICATION STEP** : The Legio pneumo/Cc r-gene[®] assay uses the same workflow than all the MWS Respiratory range assays.



BioMérieux Argene workflow followed for these studies

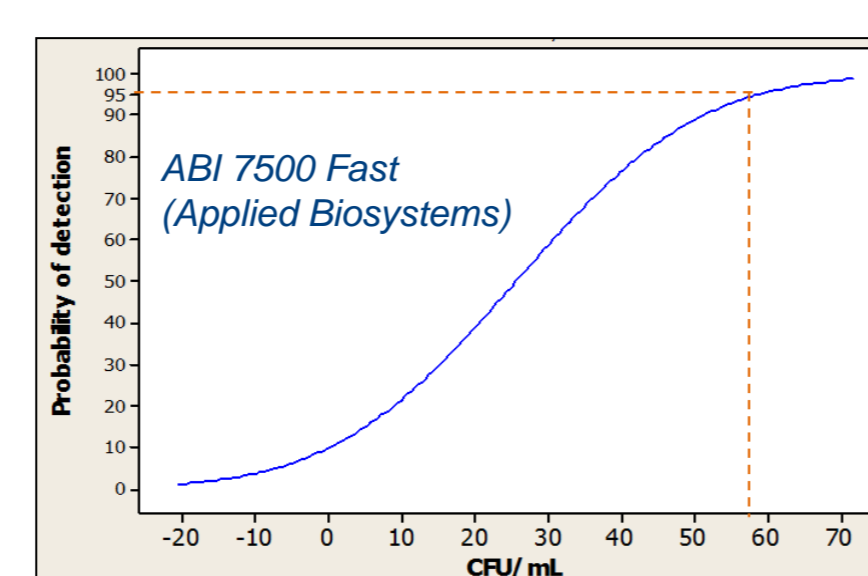
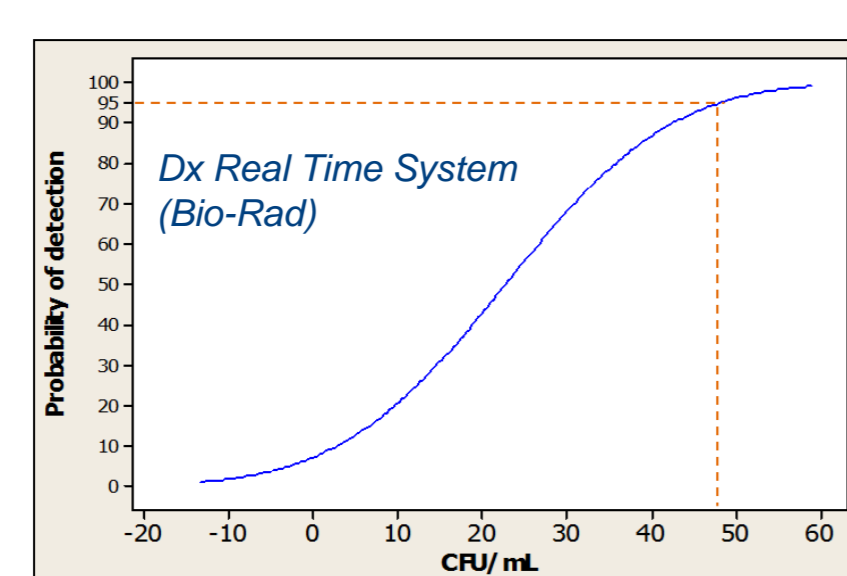
- ✓ **ANALYTICAL SENSITIVITY** : Determined on 15 replicates of 6 dilutions from a quantified *Legionella pneumophila* Sg 1 spiked in a pool of nasopharyngeal specimens.
- ✓ **REACTIVITY STUDY** : The primers and probes specificity was tested by *in silico* analysis (virus, bacteria and human sequences). The inclusivity and exclusivity were experimentally checked on 108 pathogens including one strain of each of the 15 serogroups of *Legionella pneumophila* and different strains of *Legionella non pneumophila*, viruses, bacteria, Mycobacteria and fungi. Pathogens have been tested at concentrations ranged from 10³ to 10⁶ particles/mL except for the Mycobacteria and the bacteria *Nocardia asteroides* that were not quantified.
- ✓ **PRECISION DETERMINATION** : The intra-assay and inter-assay variability studies were carried out on a quantified culture of *Legionella pneumophila* diluted in a *Legionella pneumophila*-negative respiratory sample. Samples were extracted on NucliSENS[®] easyMAG[®], with the specific B protocol, respectively 400/100 and 200/50 for intra-assay and inter-assay variability. The samples were tested 10 times in the same run on the Dx Real Time system for the intra-assay variability determination and 10 times in 10 separate experiments for the inter-assay variability determination.
- ✓ **QCMD PANEL TESTING** : *Legionella pneumophila* QCMD panels 2013 and 2014 were tested. Amplification was performed in duplicate.

RESULTS

- ✓ **ANALYTICAL SENSITIVITY** : The results obtained for the limit of detection of *Legionella pneumophila* in nasopharyngeal specimens by probit analysis using the Legio pneumo/Cc r-gene[®] kit are 48.3 CFU/mL with a CI 95% [40.4 – 61.8] on Dx Real Time system (Bio-Rad) and 58.2 CFU/mL with a CI 95% [49.7 – 71.7] on ABI 7500 Fast (Applied Biosystems).

Probit Analysis

Minitab 16



LoD 95%

48.3 CFU/mL
with a CI 95% [40.4 – 61.8]

58.2 CFU/mL
with a CI 95% [49.7 – 71.7]

CONCLUSION

The sensitivity, specificity and precision of the Legio pneumo/CC r-gene[®] kit could contribute to a better management of patient. Furthermore, the performance associated with its compatibility with the major extraction and PCR platforms and its capacity to be used in combination with others kits of the MWS r-gene[®] range allows an immediate integration of this kit into most routine diagnostic laboratories for the respiratory infections management.

- ✓ **REACTIVITY STUDY** : Tests on 108 pathogens, only *Legionella pneumophila* strains were detected.

➤ **INCLUSIVITY** : The following strains of *Legionella pneumophila* were correctly detected :

<i>Legionella pneumophila</i>	
	Lp Sg 1, Lp Sg 2, Lp Sg 3, Lp Sg 4, Lp pascullei Sg 5, Lp fraseri Sg 5, Lp Sg 6, Lp Sg 7, Lp Sg 8, Lp Sg 9, Lp 10 (environmental), Lp Sg 11, Lp Sg 12, Lp Sg 13, Lp Sg 14 (environmental), Lp Sg 15 (environmental), Lp Sg 2 (environmental), Lp Sg 1 (LrefII pept C3), Lp Sg 1 (clinical sample).

➤ **EXCLUSIVITY** : The following strains of *Legionella non-pneumophila*, bacteria, viruses, mycobacteria and fungi were not detected:

<i>Legionella non-pneumophila</i>	
	<i>Legionella bozemanii</i> , <i>Legionella dumoffi</i> , <i>Legionella longbeachae</i> , <i>Legionella feeleii</i> , <i>Legionella micdadei</i> , <i>Legionella anisa</i> , <i>Legionella cherii</i> , <i>Legionella gormanii</i> , <i>Legionella hackeliae</i>
Other bacteria	<i>Acinetobacter baumannii</i> , <i>Bordetella Bronchiseptica</i> , <i>Bordetella parapertussis</i> , <i>Bordetella pertussis</i> , <i>Branhamella catarrhalis</i> , <i>Chlamydia pneumoniae</i> , <i>Citrobacter freundii</i> , <i>Citrobacter koseri</i> , <i>Enterobacter cloacae</i> , <i>Enterobacter kobei</i> , <i>Escherichia coli</i> , <i>Haemophilus influenzae</i> , <i>Haemophilus parainfluenzae</i> , <i>Klebsiella pneumoniae</i> , <i>Klebsiella oxytoca</i> , <i>Morganella morganii</i> , <i>Mycoplasma pneumoniae</i> , <i>Nocardia asteroides</i> , <i>Proteus mirabilis</i> , <i>Pseudomonas aeruginosa</i> , <i>Raoultella ornithinolytica</i> , <i>Serratia marcescens</i> , <i>Staphylococcus aureus</i> , <i>Staphylococcus epidermidis</i> , <i>Stenotrophomonas maltophilia</i> , <i>Streptococcus agalactiae</i> , <i>Streptococcus constellatus</i>
Viruses	Herpesvirus : CMV, EBV, HSV1, 2, VZV, HHV6, 7, 8 ; Polyomavirus : BK, JC ; Bocavirus 1 ; Parvovirus B19 ; Entérovirus : Cox B2, Cox A9 ; Parechovirus 1, 2 ; Echovirus 9, 25, 30 ; Polio virus S3 ; Adenovirus : AdV3, 4, 5, 8, 11, 12, 40 ; Rhinovirus : 14, 87, 1B ; Influenza virus : A, B ; Coronavirus NL63 ; Mumps ; Parainfluenza virus : Para1, 2, 3, 4 ; Respiratory syncytial virus A, B ; Human metapneumovirus A, B.
Mycobacteria	<i>M. avium</i> , <i>M. chelonae</i> , <i>M. fortuitum</i> , <i>M. goodii</i> , <i>M. intracellulare</i> , <i>M. kansasii</i> , <i>M. lentiflavum</i> , <i>M. tuberculosis</i> , <i>M. xenopi</i>
Fungi (yeast)	<i>Candida albicans</i>

- ✓ **PRECISION DETERMINATION** : The coefficients of variation are ranged from 0.94% to 2.89% and from 1.02% to 2.37% respectively for intra-assay and inter-assay reproducibility for *Legionella pneumophila* parameter.

<i>Legionella pneumophila</i>	Average Ct (cycles)	Intra-assay variability			Inter-assay variability		
		Standard deviation	Coefficient of variation (%)	Average Ct (cycles)	Standard deviation	Coefficient of variation (%)	
10*LoD	483 CFU/mL	34.38	0.32	0.94	34.36	0.35	1.02
5*LoD	242 CFU/mL	35.83	0.81	2.26	35.43	0.53	1.50
2*LoD	97 CFU/mL	37.35	1.08	2.89	36.64	0.86	2.37
0.01*LoD	0.48 CFU/mL	Neg	Neg	Neg	Neg	Neg	Neg

- ✓ **QCMD PANEL TESTING** : On the *Legionella pneumophila* QCMD Panels 2013 and 2014, 100% (20/20) of the “Core” and “Educational” samples were correctly identified.

Samples	Sample Content (QCMD)	Sample type (QCMD)	Matrix (QCMD)	Legio pneumo/CC r-gene [®] -71-046-ABI 7500 Fast	
				Average Ct 530 nm (cycles)	Average Ct 560 nm (cycles)
LP13-01	Lp sg1	Core	STM	32.72	32.88
LP13-02	Analytical negative	Core	STM	Neg	Neg
LP13-03	Lp sg3	Core	STM	30.56	36.12
LP13-04	<i>L. bozemanii</i>	Educational	STM	Neg	Neg
LP13-05	Lp sg3	Core	STM	33.97	38.26
LP13-06	Lp sg3	Educational	STM	38.56	Neg
LP13-07	Lp sg1	Core	BAL	29.95	25.73
LP13-08	Lp sg1	Educational	BAL	36.36	25.90
LP13-09	Clinical negative	Core	BAL	Neg	26.21
LP13-10	Lp sg1	Core	BAL	33.48	25.60

Samples	Sample Content	Sample type	Matrix	Legio pneumo/CC r-gene [®] -71-046-ABI 7500 Fast	
				Average Ct 530 nm (cycles)	Average Ct 560 nm (cycles)
LP14-01	Lp sg1	Core	BAL	29.89	26.40
LP14-02	Lp sg1	Core	STM	29.95	33.95
LP14-03	Lp sg1	Educational	BAL	30.02	33.72
LP14-04	<i>L. longbeachae</i>	Educational	STM	Neg	44.26
LP14-05	Lp sg3	Core	STM	31.52	38.29
LP14-06	Lp sg1	Core	STM	33.45	38.65
LP14-07	Clinical negative	Core	BAL	Neg	26.21
LP14-08	Analytical negative	Educational	STM	Neg	43.45
LP14-09	Analytical negative	Educational	BAL	Neg	38.01
LP14-10	Lp sg1	Educational	BAL	33.36	36.51

STM: Sample Transport Medium. BAL: Bronchoalveolar Lavage