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IDENTIFICATION OF LOWER RESPIRATORY TRACT INFECTION PATHOGENS M-PNEUMONIAE, S-PNEUMONIAE AND C- PNEUMONIAE ARE COLONIZED WITH HAEMOPHILLUS INFLUENZAE USING REAL TIME PCR PLATFORM

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ABSTRACT
Bacterial respiratory tract infections have long been a challenge for the clinical microbiology laboratory. Efforts have been made to develop accurate and rapid diagnostic assays. Commercial respiratory panels currently available do not identify some important bacterial pathogens responsible for lower respiratory tract infections (LRTI). Mild LRTIs can sometimes become more severe, leading to pneumonia, bronchitis, or other more serious infections in all ages in our community. Patients with pneumoniae are colonized with *Haemophillus influenzae, Streptococcus pneumoniae, Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. At the lab, we have developed a real time PCR based detection assay for lower respiratory pathogens, *Haemophillus influenzae, Streptococcus pneumoniae, Chlamydia pneumoniae* and *Mycoplasma pneumoniae* on Quant 12 k flex diagnostic platform. The assay is designed towards detection of four targets: Autolysin (lyt A) gene *for Streptococcus pneumoniae*, L-Fuculokinase (fuc K) gene for *Haemophillus influenzae* and 16S rRNA gene *Chlamydia pneumoniae* and P1 adhesin gene for *Mycoplasma pneumoniae*. A sample processing control (SPC) Xeno is monitored along with detection of all four-target amplification. SPC control is for recovery of nucleic acid, detection of inhibitory substances and confirmation of PCR reagent integrity.

KEYWORDS
- **Sputum Sample:** a test that uses sputum, a thick mucus made in the lungs, to help check for any type of organism that can be causing an infection in the airways or the lungs. It can also be used to see if a chronic illness has worsened or to see if a treatment is working.
- **Pneumonia:** infection in which the air sacs of the lungs fill with fluid, causing fever, chills, difficulty breathing and coughing with sputum.
- **Pure culture**: a culture in which only one clone or strain is present.
- **Real-time PCR-based assay:** a method for the detection, quantification and typing of different microbial agents.
- ***Chlamydia pneumoniae:*** is an obligate intracellular bacterium that infects humans and is a major cause of pneumonia.
- **Lower Respiratory Tract infections (LRTI):** infection in the lower airways

INTRODUCTION
In the clinical microbiology laboratory, bacterial respiratory tract infections have consistently presented a formidable challenge, inspiring a concerted effort to develop precise and efficient diagnostic modalities. The variety of commercial respiratory panels currently on the market, despite advancements in diagnostic technology, is still insufficient to capture crucial bacterial pathogens responsible for lower respiratory tract infections (LRTI).[1,2] In clinical scenarios where microbiology test outcomes are pending, patients are typically prescribed broad-spectrum antibiotics. However, due to the slow, negative, or inconclusive nature of these tests, the transition to narrower-spectrum treatments is infrequently implemented in clinical practice.[3,4] LRTIs are the most common reason for consultation or admission to health-care facilities and primary care, in addition to being the deadliest infectious diseases worldwide, particularly among children and the elderly, and they are reported to have a significant impact on the increasing requests for medical examinations at both medical offices and emergency departments, antimicrobial prescriptions, and hospitalizations.[5,6] The potential development of mild LRTIs into exacerbated manifestations, such as pneumonia, bronchitis, and other infections with grave consequences across various age

strata in our community, further complicates the clinical environment.

The colonization of the respiratory tract by a group of bacteria, prominently *Haemophilus influenzae, Streptococcus pneumoniae, Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*, is a notable phenomenon seen in patients with pneumonia. Our lab has developed a cutting-edge real-time polymerase chain reaction (PCR) based detection assay, expertly calibrated for the identification of these consequential lower respiratory pathogens, in direct response to these complex challenges. An important paradigm shift in the field of diagnostic practices is being marked by the dynamic realization of this novel approach on the sophisticated Quant 12 k flex diagnostic platform. A primary advantage of the PCR technique over traditional culture methods is its reliance on amplifying the DNA or RNA of even minute microbe populations. This attribute renders it unaffected by prior antibiotic usage and imparts heightened sensitivity for detecting various bacteria, delivering swifter results.[7,8,9,10]

Precision targeting of four distinct genetic markers, including the Autolysin (lyt A) gene representative of *Streptococcus pneumoniae*, the L-Fuculokinase (fuc K) gene representative of *Haemophilus influenzae*, the 16S rRNA gene representative of *Chlamydia pneumoniae*, and the P1 adhesin gene representative of *Mycoplasma pneumoniae*, is key to the assay's effectiveness. The addition of a vigilant sample processing control (SPC), known as Xeno, which is skilled at ensuring the careful recovery of nucleic acid, identifying inhibitory agents, and validating the unimpaired integrity of the PCR reagents, strengthens the fidelity of this diagnostic platform.

METHODOLOGY

We used the LRTI assay for Nasopharyngeal swabs and sputum samples. A total of 50 throat samples were obtained from a reference laboratory for correlation studies. For LRTI assay Nasopharyngeal swabs were pretreated with lysis buffer, Pro K, Carrier RNA whereas sputum samples were pretreated with sputum liquefying reagent Snotbuster [Cpoan Italia] and Pro K. Extraction of nucleic acids from specimens was extracted using Thermo fisher viral pathogen extraction kit. All five primer probe cocktails were added 96 well plates along with real time PCR master mix. Cycling parameters for real time PCR are: 950C for 5 minutes followed by 45 cycles of 950C for 45 seconds and 600C for 45 seconds. The run time for LRTI assay is 2 h and 38 minutes. Analysis is performed on Quant studio software. Signals from probe for *H.influenzae, Streptococcus pneumoniae, Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were detected on different channels.

A sample processing control (SPC) Xeno is monitored along with detection of all four target amplification. SPC control is for recovery of nucleic acid, detection of inhibitory substances and confirmation of PCR reagent integrity.

Figures and Tables

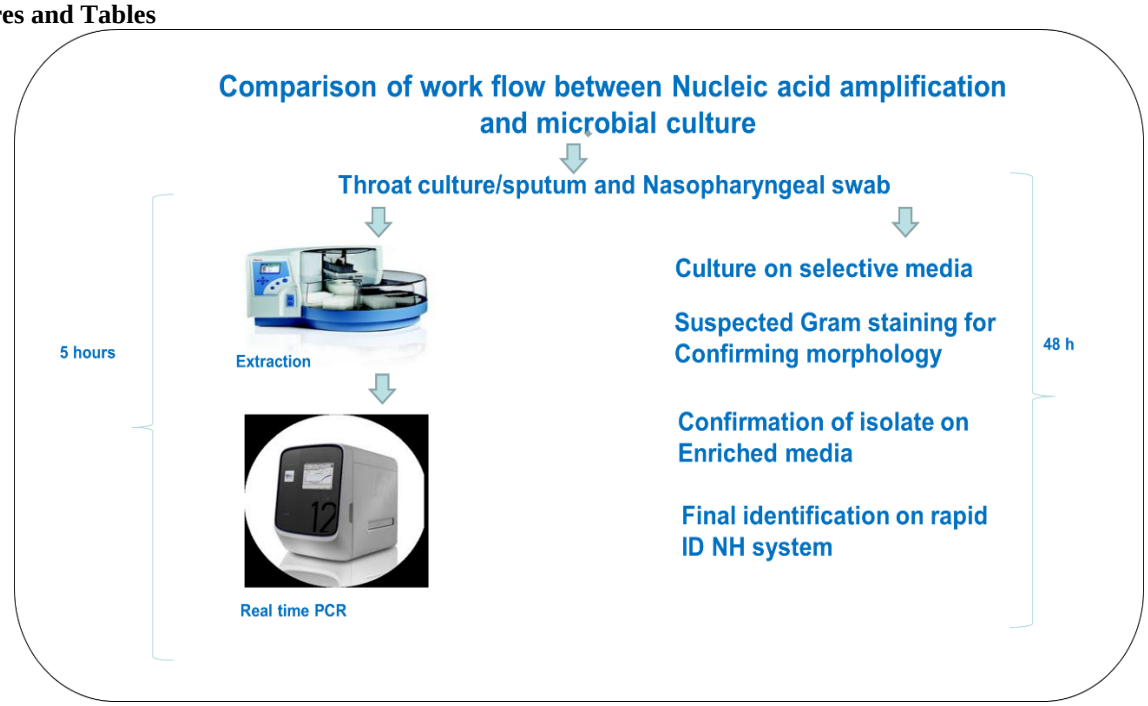

Figure 1 (above): Workflow of the LRTI array versus routine culture methods.

LRTI assay involves pretreatment of sample followed by DNA extraction and Real time PCR from Nasopharyngeal swabs and sputum samples.

Table 1 (above): Accuracy between LRTI assay and other PCR and culture method.

		Real time PCR on Quant studio		
		Real time PCR		
		POSITIVE	NEGATIVE	TOTAL
PCR and Culture	POSITIVE	34	0	34
	NEGATIVE	0	16	16
	TOTAL	34	16	50

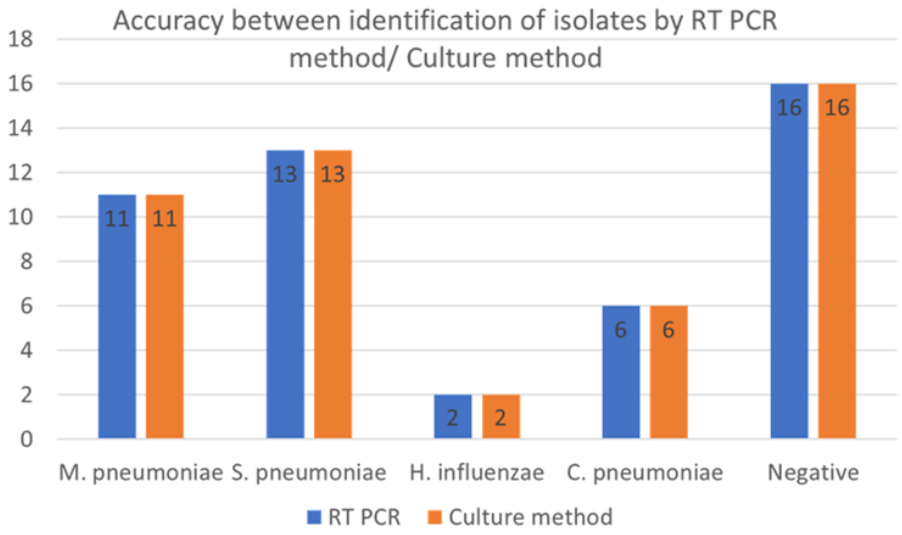

Figure 2 (above): Accuracy in isolates detected from Positive samples and Accuracy between Negative Throat samples.

Table 2 (above): Comparison between LRTI assay in isolates mixed together and when spiked in negative sputum.

Isolates spiked in sputum together [20-80,000 cfu/ml]	Mean Ct	Isolates spiked in sputum [80-100,000 cfu/ml]	Mean Ct
M. pneumoniae	29	*M. pneumoniae*	28
S. pneumoniae	29	*S. pneumoniae*	27
H. influenzae	30	*H. influenzae*	28
C. pneumoniae	28	*C. pneumoniae*	26

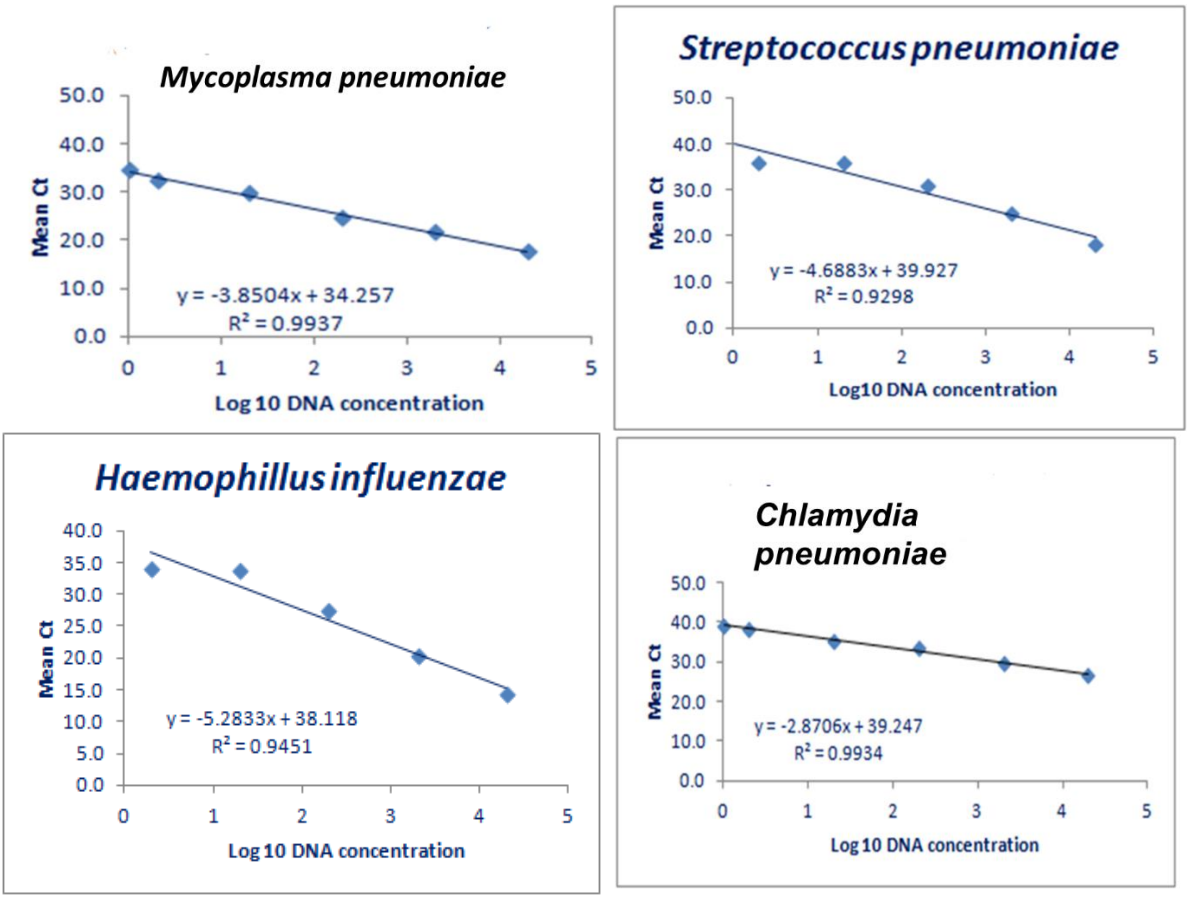

Figure 3 (all 4 graphs above): Standard curves for Respiratory pathogens. Overnight grown cultures were used for dilution to make standard curves using the LRTI assay.

Table 3 (above): Comparison of Cfu/ml and Ct values for Respiratory pathogens. Overnight grown cultures were used for dilution to make standard curves using the LRTI assay.

cfu/ml	Mycoplasma pneumoniae Mean Ct	Haemophillus influenzae Mean Ct	Streptococcus pneumoniae Mean Ct	Chlamydia pneumoniae Mean Ct
>100,000	17.7	18.1	17.3	26.7
80,000-100,000	21.8	24.8	22.5	29.7
20,000-80,000	24.8	30.9	23.6	33.4
10,000-20,000	30	35.9	29.8	35.1
<10,000	32.5	36.5	33.6	38.4
2	34.5	36.3	37.2	39.2

RESULTS

- PCR based detection assay for lower respiratory pathogens, *Haemophillus influenzae*, *Streptococcus pneumoniae*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* on Quant 12 k flex diagnostic platform was rapid, sensitive, and specific assay.
- Results observed between RT PCR and culture method were in 100% agreement.
- Primers and probes designed for target genes for each of the isolates, *Haemophillus influenzae*, *Streptococcus pneumoniae*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* were specific. Pure cultures of four pathogens were tested with all four primers and probes, there was no cross reactivity observed.
- Pure cultures of all four pathogens at a concentration of 20-80,000 cfu/ml were pooled and spiked in sputum. RT PCR assay showed good sensitivity as could detect all four pathogens.

CONCLUSION

The developed real-time PCR-based detection assay represents a significant advancement in addressing the challenges of diagnosing bacterial respiratory tract infections. The assay's accuracy and rapidity make it a promising tool for clinical microbiology laboratories. The inclusion of crucial bacterial pathogens – *Haemophilus influenzae*, *Streptococcus pneumoniae*,

Chlamydia pneumoniae, and *Mycoplasma pneumoniae* – underlines its comprehensive scope in detecting lower respiratory tract infections.

The assay's specificity is evident in its precise target gene selection, with no cross-reactivity observed in pure cultures. Its sensitivity is well-demonstrated through successful pathogen detection, even at low concentrations, affirming its efficacy in real-world scenarios. Correlation studies with conventional culture methods further underscore the assay's reliability, displaying a remarkable 100% agreement.

Operational practicality is enhanced through a relatively short run time and streamlined analysis on the Quant Studio software. This innovation has the potential to significantly impact patient care and public health by enabling early and accurate identification of bacterial pathogens responsible for respiratory infections. As such, this real-time PCR assay stands as a valuable asset in the ongoing efforts to mitigate the challenges posed by bacterial respiratory tract infections.

ACKNOWLEDGMENTS

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Conflicts of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Macfarlane J, Holmes W, Gard P, Macfarlane R, Rose D, Weston V, et al. Prospective study of the incidence, aetiology and outcome of adult lower respiratory tract illness in the community. *Thorax*, 2001; 56(2): 109–114.
2. Reimer LG, Carroll KC. Role of the microbiology laboratory in the diagnosis of lower respiratory tract infections. *Clin Infect Dis.*, 1998; 26(3): 742–748.
3. Gadsby, N. J., McHugh, M. P., Russell, C. D., Mark, H., Conway Morris, A., Laurenson, I. F., Hill, A. T., & Templeton, K. E. Development of two real-time multiplex PCR assays for the detection and quantification of eight key bacterial pathogens in lower respiratory tract infections. Clinical Microbiology and Infection, 2015; 21(8). https://doi.org/10.1016/j.cmi.2015.05.004
4. Public Health England. Investigation of bronchoalveolar lavage, sputum and associated specimens. UK Standards for Microbiology Investigations, 2014; B57: 2.5. http://www.hpa.org.uk/SMI/pdf.
5. WHO Severe Acute Respiratory Infections Treatment Centre. *Practical Manual to Set Up and Manage a SARI Treatment Centre and a SARI Screening Facility in Health Care Facilities.* World Health Organization; Geneva, Switzerland, 2020; 120.
6. Yanagihara K. The Role of Molecular Diagnosis in Acute Respiratory Tract Infection. *Respir. Investig*, 2019; 57: 511. doi: 10.1016/j.resinv.2019.06.007
7. Murdoch DR. Impact of rapid microbiological testing on the management of lower respiratory tract infection. *Clin Infect Dis.*, 2005; 41(10): 1445–1447.
8. Luo YC, Du P, Zhao JZ, Duan XJ, Hou YJ, Pan H, et al. A multiplex touchdown PCR for detection of Streptococcus pneumoniae, Haemophilus influenzae type b and Mycobacterium tuberculosis complex in sputum samples. *Trop Biomed*, 2012; 29(3): 422–428.
9. Pozzetto B, Grattard F, Pillet S. Multiplex PCR theranostics of severe respiratory infections. *Expert Rev Anti Infect Ther.*, 2010; 8(3): 251–253. doi: 10.1586/eri.09.131.
10. Gimferrer L, Andrés C, Rando A, Piñana M, Codina MG, Martin MDC, *et al*. Evaluation of Seegene Allplex Respiratory Panel 1 kit for the detection of influenza virus and human respiratory syncytial virus. J Clin Virol., 2018; 105: 31-4.