Addressing pulmonary nocardiosis risk in immunocompromised patients: Development and validation of a commercially available PCR

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INTRODUCTION

Current state of diagnosis of pulmonary nocardiosis. Pulmonary nocardiosis is an infection targeting immunocompromised patients that is characterized by high mortality and requires non-frontline antibiotics for treatment, such as linezolid and trimethoprim/sulfamethoxazole (Martinez T et al., 2007). Early diagnosis of *Nocardia* infections remains a major challenge. Difficulties persist in identifying *Nocardia* due to extended culture times for BAL.

Importance of early diagnosis for nocardiosis. Nocardiosis is currently confirmed or excluded by BAL fluid culture followed by further phenotypic identification steps. A culture-independent method with more timely results would accelerate the administration of appropriate treatment (Wilson JW, 2012). A rapid Nocardia PCR assay for BAL has neither been validated nor offered for clinical testing to our knowledge.

Early detection of nocardiosis aided by rapid molecular testing. This commercially available real-time PCR assay may permit early detection of nocardiosis and administration of appropriate treatment in a timely manner, when compared to the culture method.

MATERIALS AND METHODS

A rapid and sensitive real-time PCR assay was developed by Viracor Eurofins Clinical Diagnostics for detection of *Nocardia* species by TaqMan technology for use in BAL specimens. Oligonucleotides for a *Nocardia* PCR were aligned to the 16S regions of common causative agents of nocardiosis and over 30 others (Brown-Elliot BA et al., 2012). The target species include: *N. abscessus, N. africana, N. asteroides, N. brasiliensis, N. cyriacigeorgica, N. farcinica, N. nova, N. otitidiscaviarum, N. pseudobrasiliensis, N. transvalensis*, and *N. wallacei*. The assay has one fluorogenic TaqMan probe that is FAM-labeled and thus detects, but does not distinguish *Nocardia* species (**Table 1**). Rapid automated nucleic acid extraction (<1 hour for 24 samples) followed by fast PCR (<1 hour) was validated according to relevant compliance standards at Viracor Eurofins (**Figure 1**). Spiked and un-spiked human BAL samples were used to assess analytical specificity, limit of detection, precision and accuracy using commercially available *Nocardia* (**Table 2**) and non-*Nocardia* strains (**Table 3**).

Table 1: *Nocardia* PCR Assay Oligonucleotides

Assay Oligonucleotides	Number
Nocardia Forward Primer	1
Nocardia Reverse Primer	1
Nocardia Probes (FAM)	4

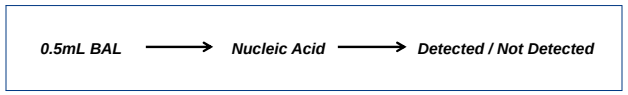

Figure 1. Nocardia Real-Time PCR Assay Workflow

RESULTS

Table 2. Analytical Specificity: Inclusivity

Detected		Detected in-Silico	
• *Nocardia abscessus*	• *Nocardia nova*	• *Nocardia puris*	• *Nocardia crassostraea*
• *Nocardia africana*	• *Nocardia otitidiscaviarum*[1]	• *Nocardia cerradoensis*	• *Nocardia thailandica*
• *Nocardia asteroides*	• *Nocardia pseudobrasiliensis*	• *Nocardia vulneris*	• *Nocardia brevicatena*
• *Nocardia brasiliensis*	• *Nocardia transvalensis*	• *Nocardia tenerifensis*	• *Nocardia higoensis*
• *Nocardia cyriacigeorgica*	• *Nocardia wallacei*[1]	• *Nocardia araoensis*	• *Nocardia harenae*
• *Nocardia farcinica*		• *Nocardia testacea*	• *Nocardia carnea*
		• *Nocardia arthritidis*	• *Nocardia paucivorans*
		• *Nocardia beijingensis*	• *Nocardia asiatica*
		• *Nocardia mexicana*	• *Nocardia vinacea*
		• *Nocardia amamiensis*	• *Nocardia aobensis*

[1] *N. otitidiscaviarum* and *N. wallacei* were detected below the LOD of the assay.

Table 3. Analytical Specificity: Cross-Reactivity

Detected[1]	Not Detected	
• *Crossiella cryophila*	• *Acinetobacter baumannii*	• *Mycoplasma pneumoniae*
• *Rhodococcus equi*	• *Actinomyces israelii*	• *Neisseria gonorrhoeae*
	• *Arcanobacterium haemolyticum*	• *Neisseria meningitidis*
	• *Bordetella holmesii*	• *Pseudomonas aeruginosa*
	• *Bordetella parapertussis*	• *Staphylococcus aureus*
	• *Bordetella pertussis*	• *Streptococcus dysgalactiae*
	• *Chlamydia pneumoniae*	• *Streptococcus pneumoniae*
	• *Chlamydia trachomatis*	• *Streptococcus pyogenes*
	• *Corynebacterium diphtheriae*	• *Saccharothrix longispora*
	• *Fusobacterium necrophorum*	• *Nocardiopsis dassonvillei subsp. dassonvillei*
	• *Haemophilus influenza Type B*	• *Nocardiopsis dassonvillei subsp. albirubida*
	• *Klebsiella pneumoniae*	
	• *Legionella pneumophila*	
	• *Moraxella catarrhalis*	
	• *Mycobacterium bovis*	
	• *Mycobacterium microti*	
	• *Mycobacterium tuberculosis*	

[1] Two non-*Nocardia* species were detected at low levels when amounts of the organism were tested at the highest concentrations permitted by available bacterial stocks. *Rhodococcus equi* and *Crossiella cryophila* have had >100 and zero reported cases in literature, respectively.

Table 4. Limit of Detection

Nocardia species	Specimen type	Copies per mL BAL
Nocardia cyriacigeorgica[1]	BAL	206
Nocardia nova[2]	BAL	41
Nocardia transvalensis[2]	BAL	26

[1]The limit of detection for *N. cyriacigeorgica* was determined by testing 10-20 replicates of human BAL spiked at 9 levels of *N. cyriacigeorgica*. A total of 220 replicates were extracted and assayed by *Nocardia* PCR.
[2]A partial limit of detection for *N. nova* and *N. transvalensis* was performed, as these, along with *N. cyriacigeorgica*, are common causative agents of nocardiosis. The results were analyzed by Probit to predict the limit of detection, which is defined here as the concentration at which 95% of replicates are detected.

RESULTS

Table 5. Qualitative Analytical Cutoff

Nocardia species	Specimen type	Copies per mL BAL
Nocardia cyriacigeorgica[1]	BAL	206
Nocardia nova	BAL	41
Nocardia transvalensis	BAL	26

Note. Qualitative analytical cutoff was determined from Probit analyses from Limit of Detection data (Table 4). Qualitative analytical cutoff is calculated as the C_{50} upper 95% confidence interval for each of the three species tested.

Table 6. Precision: Intra- and Inter-Assay Reproducibility

Precision	BAL	
	High Concentration (200,000 copies/mL)	Low Concentration (2,000 copies/mL)
Intra % CV	<10%	<8%
Inter % CV	<21%	<26%

Note. Five replicates of low and high concentrations of *N. cyriacigeorgica* spiked in human BAL were analyzed over three experimental days by multiple operators / instruments. The % coefficient of variation (%CV) was determined using the mean and standard deviation values of the copies/mL.

Table 7. Analytical Accuracy

Analytical Accuracy	BAL	
	Positive	Negative
Detected	100% (33/33)	0% (0/11)
Not Detected	0% (0/33)	100% (11/11)

Note. Target species listed in the methods were spiked in human BAL in triplicate, and 11 un-spiked samples were analyzed for accuracy.

CONCLUSIONS

The specificity, inclusivity, sensitivity, precision and accuracy of a qualitative Real-time *Nocardia* PCR have been deployed as an aid in the diagnosis of pulmonary nocardiosis.

Nocardia PCR allows for a culture-independent method that can rapidly detect clinically relevant *Nocardia* species with an improved turnaround time, leading to prompt diagnosis and administration of appropriate treatment.

References

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