

Development of a Multiplex Real-Time PCR Assay for the Simultaneous Detection of Three Fungal Pathogens in respiratory samples

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Background

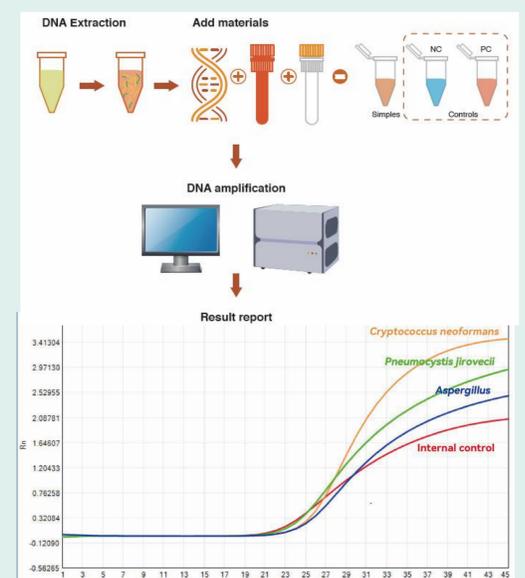
- Approximately 6.5 million new cases of invasive fungal infections (IFI) and 3.8 million deaths are reported annually, affecting over 1 billion people worldwide. *Aspergillus*, *Cryptococcus*, and *Pneumocystis* are the common pathogens leading to IFI.
- **In this study, a multiple real-time polymerase chain reaction (PCR) assay was developed to simultaneously detect and identify three of the most frequent pathogens in sputum and bronchoalveolar lavage fluid (BALF) samples, including *Aspergillus*, *Cryptococcus neoformans*, and *Pneumocystis jirovecii*, and to evaluate its performance on clinical samples.**

Methods

- Sputum samples (n=244) and BALF samples (n=299) from patients with suspected IFI were collected from three centres. The diagnostic sensitivity and specificity of the multiplex real-time PCR assay were analysed.
- DNA sequencing was used as the reference method, and the performance of the multiplex real-time assay was evaluated by determining the positive percent agreement (PPA), negative percent agreement (NPA), and overall coincidence rate.
- The reproducibility, limit of detection (LoD), and interference experiment of the multiplex real-time PCR assay were also evaluated.

Conclusions

The multiplex real-time PCR assay was established with high sensitivity and specificity for rapid and simultaneous detection on three common fungal pathogens in respiratory tract samples.



Results

- From the clinical evaluation results, the sensitivity and specificity of the multiplex real-time PCR assay were greater than 90% for *Aspergillus*, *Cryptococcus neoformans*, and *Pneumocystis jirovecii* (Table 1).
- The consistency of the multiplex real-time PCR assay and DNA sequencing was almost perfect: the overall coincidence rate of the two methods was close to 100% (Table 2).
- The percent coefficient variations (CVs%) of reproducibility was less than 5%, meeting requirements, and the LoD of the kit for each of the three species less than or equal to 1000 copies/mL (Table 3).
- No cross-reactivity was detected for any bacteria or fungi.

Table 1 Clinical performance of the multiplex real-time PCR assay

Sample type	Target Organism	Sensitivity (%)	Specificity (%)
Sputum	<i>Aspergillus</i>	94.94 (75/79)	97.59 (81/83)
	<i>Cryptococcus neoformans</i>	90.91 (20/22)	96.39 (80/83)
	<i>Pneumocystis jirovecii</i>	92.50 (74/80)	96.39 (80/83)
BALF	<i>Aspergillus</i>	97.06 (132/136)	100.00 (58/58)
	<i>Cryptococcus neoformans</i>	92.00 (46/50)	98.28 (57/58)
	<i>Pneumocystis jirovecii</i>	94.55 (52/55)	98.28 (57/58)

Table 2 Comparison between the multiplex real-time PCR assay and DNA sequencing results in sputum and BALF

Sample type	Target Organism	Positive percent agreement (%)	Negative percent agreement (%)	Overall percent agreement (%)
Sputum	<i>Aspergillus</i>	100.00 (79/79)	100.00 (83/83)	100.00 (162/162)
	<i>Cryptococcus neoformans</i>	95.45 (21/22)	100.00 (83/83)	99.05 (104/105)
	<i>Pneumocystis jirovecii</i>	97.50 (78/80)	100.00 (83/83)	98.77 (161/163)
BALF	<i>Aspergillus</i>	100.00 (136/136)	100.00 (58/58)	100.00 (194/194)
	<i>Cryptococcus neoformans</i>	98.00 (49/50)	100.00 (58/58)	99.07 (107/108)
	<i>Pneumocystis jirovecii</i>	100.00 (55/55)	100.00 (58/58)	100.00 (113/113)

Table 3 Verification of the LoD of the multiplex real-time PCR assay

Sample type	Target Organism	LoD (1000 copies/mL)
Sputum	<i>Aspergillus</i>	100% (20/20)
	<i>Cryptococcus neoformans</i>	95% (19/20)
	<i>Pneumocystis jirovecii</i>	100% (20/20)
BALF	<i>Aspergillus</i>	100% (20/20)
	<i>Cryptococcus neoformans</i>	100% (20/20)
	<i>Pneumocystis jirovecii</i>	100% (20/20)