

Evaluation of the Performance of a Multiplex Real-Time PCR Assay for the Identification of *Aspergillus*, *Cryptococcus neoformans*, and *Pneumocystis jirovecii* Simultaneously from Sputum in Multicenter [Response to Letter]

Wenjing Liu¹, Yajie Wang², Hongli Sun¹

¹Department of Laboratory Medicine, State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing Key Laboratory for Mechanisms Research and Precision Diagnosis of Invasive Fungal Diseases, Beijing 100730, People's Republic of China; ²Department of Clinical Laboratory, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, People's Republic of China

Correspondence: Hongli Sun, Peking Union Medical College Hospital (Dongdan Campus), No. 1 Shuaifuyuan Wangfujing Dongcheng District, Beijing, 100730, People's Republic of China, Tel +86-1069159788, Fax +86-1069159766, Email sunhl2010@sina.com; Yajie Wang, Department of Clinical Laboratory, Beijing Ditan Hospital, Capital Medical University, Beijing, 100015, People's Republic of China, Tel +86-13611269270, Email wangyajie@ccmu.edu.cn

Dear editor

We thank Idrus and Sunarno for their interest in guiding us to their recently published study, "Evaluation of the Performance of a Multiplex Real-Time PCR Assay for the Identification of *Aspergillus*, *Cryptococcus neoformans*, and *Pneumocystis jirovecii* Simultaneously from Sputum in Multicenter".¹ We have carefully reviewed their comments and attempted to answer their queries as much as possible.

Several nucleic acid test methods have been developed to identify pathogens. The MCDA-LFB is a simple, fast, reliable, and sensitive diagnostic method that can identify pathogens in basic and clinical laboratories.² The study has reported that the MCDA-LFB has a better detection ability than the PCR method.³ Indeed, the PCR method outperforms the MCDA-LFB in several ways.

Though the sensitivity of the MCDA-LFB method is 10 fg/μL for pathogens, but it's for a single pathogen. Due to the complexity and large amount of primers needed to target each gene, MCDA-LFB cannot detect multiple pathogens simultaneously. In contrast, multiplex PCR, with simple primer and probe design, allows for the detection of three to six pathogens in general and even dozens of pathogens simultaneously.^{4,5}

Then, the entire process of MCDA-LFB can be controlled within 60 minutes, if the technique is used for high-throughput detection. However, it needs a lot of manual work or complex equipment. However, the multiplex PCR only requires a PCR amplifier which is now commercially available for single-person to high-throughput automation.⁶

Finally, MCDA-LFB requires the amplification product to be tested with the lid open, which causes the contamination problem. In contrast, the multiplex PCR is airtight throughout the test which can better avoid contamination.

With the broad establishment of PCR instruments in molecular laboratories, their application in pathogens detection are more widely in the post-epidemic era. We will also keep an eye on the future development of MCDA-LFB. We thank Idrus and Sunarno for their correspondence and look forward to further discussions toward improving the application of nucleic acid test methods.

Disclosure

The authors declare no conflicts of interest for this communication.

References

1. Idrus HH, Sunarno. Evaluation of the Performance of a Multiplex Real-Time PCR Assay for the Identification of *Aspergillus*, *Cryptococcus neoformans*, and *Pneumocystis jirovecii* Simultaneously from Sputum in Multicenter [Letter]. *Infect Drug Resist.* 2022;15:6799–6800. doi:10.2147/IDR.S396184
2. Jiang L, Gu R, Li X, et al. Multiple Cross Displacement Amplification Coupled with Lateral Flow Biosensor (MCDA-LFB) for rapid detection of *Legionella pneumophila*. *BMC Microbiol.* 2022;22(1):20. doi:10.1186/s12866-021-02363-3
3. Wang Y, Yan W, Wang Y, et al. Rapid, sensitive and reliable detection of *Klebsiella pneumoniae* by label-free multiple cross displacement amplification coupled with nanoparticles-based biosensor. *J Microbiol Methods.* 2018;149:80–88. doi:10.1016/j.mimet.2018.05.003
4. Gago S, Esteban C, Valero C, et al. A multiplex real-time PCR assay for identification of *Pneumocystis jirovecii*, *Histoplasma capsulatum*, and *Cryptococcus neoformans*/*Cryptococcus gattii* in samples from AIDS patients with opportunistic pneumonia. *J Clin Microbiol.* 2014;52(4):1168–1176. doi:10.1128/JCM.02895-13
5. Zhang J, Yang F, Sun Z, et al. Rapid and precise identification of bloodstream infections using a pre-treatment protocol combined with high-throughput multiplex genetic detection system. *BMC Infect Dis.* 2022;22(1):823. doi:10.1186/s12879-022-07793-6
6. Nörz D, Hoffmann A, Aepfelbacher M, et al. Clinical evaluation of a fully automated, laboratory-developed multiplex RT-PCR assay integrating dual-target SARS-CoV-2 and influenza A/B detection on a high-throughput platform. *J Med Microbiol.* 2021;70(2):001295. doi:10.1099/jmm.0.001295

Dove Medical Press encourages responsible, free and frank academic debate. The content of the Infection and Drug Resistance 'letters to the editor' section does not necessarily represent the views of Dove Medical Press, its officers, agents, employees, related entities or the Infection and Drug Resistance editors. While all reasonable steps have been taken to confirm the content of each letter, Dove Medical Press accepts no liability in respect of the content of any letter, nor is it responsible for the content and accuracy of any letter to the editor.

Infection and Drug Resistance

Dovepress

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>

<https://doi.org/10.2147/IDR.S400931>