

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]

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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 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.

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