

Application Brief

Multiplex Fungal Panel

Unique All-In-One-Well Assay for Simultaneous Detection and Differentiation of Fungal Pathogens Directly from Whole Blood

INTRODUCTION

Invasive fungal infections have become a major cause of morbidity and mortality over the past three decades. Organ transplantation, aggressive chemotherapy, and widespread use of immunosuppressive treatments for many medical conditions have increased the risk of fungal diseases. Early and accurate diagnosis of fungal infection will drastically reduce mortality rate among infected individuals. Additionally, simultaneous detection and discrimination of these fungal pathogens will enable early and appropriate regimen thus reducing health associated risk by fungal pathogens. To address this market need and improve the patient care, PrimeradX has developed a unique panel for detection and differentiation of multiplexed targets with high performance from a range of infectious disease organisms. The multiplex capability of the ICEPlex® system also enables the detection of built-in calibrators and controls along with the fungal targets of interest.

The PrimeradX Multiplex Fungal Panel can simultaneously detect and differentiate nine major fungal pathogens in a single reaction:

- *Candida albicans*
- *Candida tropicalis*
- *Candida parapsilosis*
- *Candida glabrata*
- *Candida krusei*
- *Cryptococcus neoformans*
- *Aspergillus flavus*
- *Aspergillus fumigatus*
- *Aspergillus niger*

SUMMARY

- Detects and discriminates multiple targets in a single reaction along with necessary extraction, sensitivity, and calibration controls.
- Demonstrates proven performance on sensitivity and specificity.
- Utilizes sample extraction method that works for *Candida*, *Cryptococcus*, and *Aspergillus*.
- Reduces labor and improves lab operation.
- Expedites sample turn-around time to less than 4 hours.

METHOD HIGHLIGHTS

1. Primers were designed using PrimeradX's strategy that can selectively amplify unique genomic region for each target in the assay. One of the primers in each primer set was labeled with FAM dye.
2. Purified microbial genomic DNAs were obtained from a commercial source and a small number of clinical isolates were used to generate proof of concept data.
3. Sample extraction method was developed in-house and was used to perform analytical studies and to test clinical isolates.
4. The Multiplex Fungal Panel includes extraction and sensitivity controls.
5. Multiplex PCR reactions were subjected to thermocycling in a standard 96-well PCR plate on the ICEPlex system.
6. Fluorescently labeled amplicons were then automatically injected, separated and detected in the capillary electrophoresis module of the ICEPlex system.
7. Amplification curves for all targets and controls were generated automatically by the ICEPlex software and cycle thresholds (Cts) were calculated.

TYPICAL DATA

The Multiplex Fungal Panel can simultaneously detect and differentiate nine major fungal pathogens including *Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. krusei*, *Cryptococcus neoformans*, *Aspergillus flavus*, *A. fumigatus*, and *A. niger*. In order to achieve high sensitivity, primers were designed to target multi-copy regions on chromosomal or mitochondrial fungal genomes.

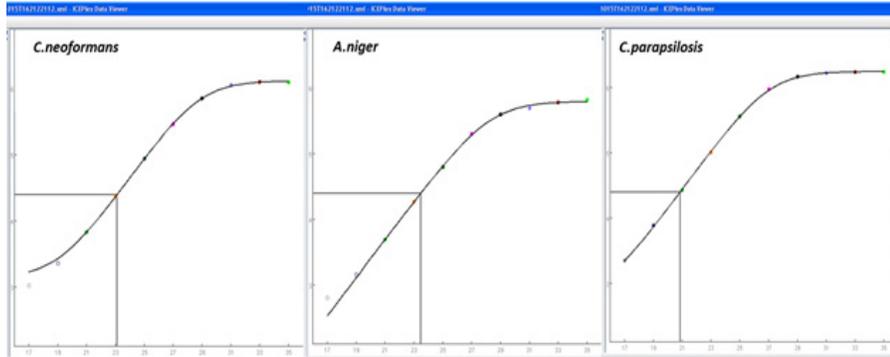


Figure 1. Representative amplification curves for 3 Multiplex Fungal Panel targets on ICEPlex system.

		<i>Candida albicans</i>	<i>Candida tropicalis</i>	<i>Candida parapsilosis</i>	<i>Candida glabrata</i>	<i>Candida krusei</i>	<i>Cryptococcus neoformans</i>	<i>Aspergillus flavus</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>
Sample 1	Ct						23.1			
	Result	No	No	No	No	No	Detected	No	No	No
Sample 2	Ct									23.5
	Result	No	No	No	No	No	No	No	No	Detected
Sample 3	Ct			20.8						
	Result	No	No	Detected	No	No	No	No	No	No

Table 1. Representative results for three samples on ICEPlex system.

FOR MORE INFORMATION

For a list of publications and to find out more about how PrimeraDx can help your lab, please contact us at 508.618.2300 or visit www.primeradx.com.

The ICEPlex system and ICEPlex Fungal Assay are for Research Use Only and have not been approved for in vitro diagnostic use by the FDA. The presented information is for demonstration purposes only.