BIOCODE® FUNGAL PANEL

Application Note AP-052023

Applied BioCode

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Introduction

According to the Centers for Disease Control and Prevention (CDC), approximately 1.5 million people in the United States are affected by fungal infections each year. Fungal infections can range from mild to severe and can affect people in many ways. Mild fungal infections may cause skin irritation, itching, and redness. Invasive Fungal Infection can cause more serious symptoms such as fever, chills, and difficulty breathing. Certain individuals, especially those with weakened immune systems, can develop invasive mold infections days to weeks after exposure to fungi that live in the environment. These infections are typically caused by *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Pneumocystis jirovecii*, endemic dimorphic fungi and Mucormycetes. Invasive fungal infection is a major cause of morbidity and mortality in immunocompromised patients.

Techniques for the diagnosis of invasive fungal infections remain reliant on antigen testing, culturing, and radiological imaging. However, these techniques suffer from prolonged turnaround times and low accuracy, which exacer-bate the challenge in the fight to prevent invasive fungal infections.

BioCode® Fungal Panel with Barcoded Magnetic Beads (BMBs) and the MDx-3000 System

The **BioCode® Fungal Panel** (Research Use Only) is a powerful tool for researchers to detect fungal species. This panel provides a comprehensive set of assays to detect and differentiate between different fungal species. The panel is designed to be used in a variety of research applications. The PCR assays offer several features that could overcome current shortcomings associated with identification of fungal infections. These PCR assays have detection limits of a few gene copies per reaction, providing the ability to detect a fraction of an organism when targeting genes present in multiple copies per fungal genome.

BioCode® Fungal Panel Targets

MOLD	
Aspergillus fumigatus	*Cunninghamella bertholletiae*
Aspergillus terreus	*Lichtheimia corymbifera*[a]
Aspergillus flavus	*Scedosporium apiospermum*
Aspergillus niger	*Lomentospora prolificans*
Mucor indicus	*Fusarium oxysporum*
Mucor circinelloides/racemosus	*Fusarium solani*
Rhizopus	*Syncephalastrum racemosum*[b]
Rhizomucor	*Pneumocystis jirovecii*

DIMORPHIC FUNGI	YEASTS
Blastomyces dermatitidis	*Cryptococcus neoformans/gattii*
Coccidioides immitis/posadasii	
Histoplasma capsulatum	

[a]*Assay may also detect some strains of other Lichtheimia species, such as L. ramosa.*

[b]*Assay may also detect some strains of other Syncephalastrum species, such as S. monosporum*

Fungal targets can be detected by PCR by first extracting the DNA from the sample. This can be done with a lysis buffer and bead beating to break down the cell walls and release the DNA. Once the DNA is extracted, it can be amplified using PCR primers that are specific to the target's DNA code. Then, during hybridization, the DNA sequences spontaneously pair to the complementary DNA through hydrogen bonding to create a double-stranded molecule. The MDx-3000 System will analyze the results providing an easy-to-read report that shows if the target is present in the sample or not.

MATERIALS AND WORKFLOW

Materials Needed

Part number	Component	Concentration	Volume/Quantity
14-M0001	BioCode Master Mix A (RUO)	2.5X	500 µL (2 vials)
24-F0006	BioCode Fungal Primer Mix (RUO)	2.5X	500 µL (2 vials)
24-F0007	BioCode Fungal BMB-Probe Mix (RUO)	100 BMB/µL	6 mL (1 vial)
23-D0001	BioCode DNA-IC (RUO)	T4 Phage	500 µL (2 vials)

Required but not provided in the Fungal Panel Kit

Part number	Component	Concentration	Volume/Quantity
63-S0001	BioCode SA-PE Mix	50 µg/mL	450 µL (8 vials)
44-B0003	BioCode Buffer A	1X	1 L (1 bottle)
01-B0010 *Bertin P000915-LYSK0-A.0*	Bertin SK38 Soil Grinding Tubes	N/A	1 pack of 50

Applied BioCode Fungal Panel General Workflow Example:

Note: *It is strongly recommended that sample preparation be performed in a biosafety cabinet with gloves and appropriate personal protective equipment (PPE)*

Sample Extraction

Pretreat Sample Method	Sample volume	1X PBS volume	DNA-IC volume	Vortex time
	200 µL	400 µL	10 µL	10 min

Step 1: Add 200 µL of each fungal sample, 400 µL 1x PBS, and 10 µL DNA-IC into each SK38 tube and perform bead beating method according to the table above.

Step 2: After vortexing for 10 minutes, spin down the SK38 tubes at 3500 rpm for 2 minutes.

Step 3: Use up to 300 µL of supernatant for extraction.

Step 4: Perform extraction.

FUNGAL PANEL PCR PLATE SET UP

Note: Prepare the PCR Plate in a dedicated reaction mix prep area

Step 1: Thaw Primer Mix, Master Mix and BMB-Probe Mix at room temperature. Perform a quick vortex (2-3 seconds) and centrifuge to collect reagents at the bottom of the tube.

Step 2: Prepare the reaction mix in a polypropylene microcentrifuge tube as described below:

			Reaction Mix	
	stock	**final**	**1 rxn**	**26 rxns**
BioCode Master Mix A	2.5 X	1.0 X	10.0 µL	260.0 µL
2.5x Fungal RUO Primer Mix	2.5 X	1.0 X	10.0 µL	260.0 µL
Template	N/A	N/A	5.0 µL	N/A
Final Volume			**25.00 µL**	**520.00 µL**

Step 3: Mix reaction mix by pipetting up and down 8 to 10 times and centrifuge to collect contents at the bottom of the tube. Store at 2-8˚C or on a cool block until ready to set up PCR (not to exceed one hour).

Step 4: Pipette 20 µL of reaction mix into appropriate wells of a 96-well plate.

Step 5: Pipette 5 µL of each extracted sample into the wells.

Step 6: Pipette 5 µL extracted negative control into the NC well.

Step 7: Seal plate with pierceable foil. Store at 2-8˚C or on a cool block until ready to load onto the BioCode MDx-3000 **(not to exceed one hour from the time the reaction mix is prepared).**

Step 8: Briefly centrifuge plate to collect samples at the bottom of the plate.

MDx-3000 Run

Step 9: Load plate onto BioCode MDx-3000. Vortex thawed room temperature BMB-Probe Mix for 30 seconds at high speed and load onto the BioCode MDx-3000. *(Note: Precipitates may appear at cold temperatures. If precipitants are present, allow the BMB-Probe Mix to warm to room temperature and vortex additional 30 seconds.)*

Step 10: Load reagents and consumables as prompted by graphic user interface and run BioCode Fungal RUO Panel according to the table below:

Run UDM on MDx-3000 using the following protocol:

	MDx-3000			
PCR Thermocycling	UNG Digestion	30˚C	5 mins	
	Initial Denaturation	95˚C	2 mins	
	Denature	95˚C	10 sec	45 Cycles
	Anneal	58˚C	25 sec	
	Extension	72˚C	15 sec	
Post-PCR Steps	**Hybridization Reaction Conditions**			
	Denature RT-PCR Amplicon	95˚C	1 mins	-
	Add 5 uL of PCR product to appropriate wells			
	Hybridization (8X SSPE)	50˚C	30 mins	
	Wash 2X			
	SAPE (50 µL)	50˚C	10 mins	
	Wash 3X **Add 250 µL of Buffer A** **Perform Optical Detection**			

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