



# Candida Albicans Drug Resistance Panel

## Use of the *Candida albicans* Drug Resistance (CaDR) Panel (ATCC® MP-8™) in antifungal drug testing

### Abstract

This study will demonstrate the use of the ATCC *Candida albicans* Drug Resistance (CaDR) Panel (ATCC® MP-8™) in antifungal drug testing.

### Introduction

*Candida* species are the fourth leading cause of hospital-acquired fungal infections among immunocompromised individuals in the United States<sup>1</sup>. Existing therapies include treatment with antifungal drugs such as echinocandins, triazoles, or pyrimidine analogs. However, due to both natural and societal selective pressures, the number of antifungal drug-resistant strains is steadily increasing. This is problematic for the treatment of fungal pathogens, such as *C. albicans*, as there are a limited number of effective antifungal drugs currently available<sup>2</sup>. Here, we investigate a panel of 12 antifungal drug resistant *C. albicans* strains for use as reference standards in the analysis of potential new antifungal drug therapies.

### Materials and Methods

To assess the molecular identity of the twelve antifungal resistant *C. albicans* strains (64124™, 10231™, 76485™, 28121™, 90819™, MYA-1023™, MYA-427™, MYA-574™, 38289™, 11651™, 96901™, and 90029™) and 2 sensitive control strains (MYA-2876™ and 18804™) within the MP-8 panel, each strain was analyzed for sequence variations in the hypervariable D1D2 region of the large subunit ribosomal RNA (LSU rRNA) gene. Strains were also examined for antifungal susceptibility using the Sensititre® YeastOne® YO9 colorimetric microdilution susceptibility test (TREK Diagnostic Systems, Cleveland, OH), according to the manufacturer's instructions. *C. albicans* strains were prepared in YeastOne® inoculum broth at an inoculum of 1.5–1.8 x 10<sup>3</sup> CFU/mL and were incubated at 35°C for 24 to 48 hours in the presence of Anidulafungin (0.015–8 µg/mL), Micafungin (0.008–8 µg/mL), Caspofungin (0.008–8 µg/mL), 5-Flucytosine (0.06–64 µg/mL), Voriconazole (0.008–8 µg/mL), Itraconazole (0.015–16 µg/mL), or Fluconazole (0.12–256 µg/mL). Yeast growth was evident as a change in the colorimetric growth indicator from blue (negative) to red (positive).

### Results and Discussion

The molecular identity of each strain was determined by sequencing the D1D2 region of the LSU rRNA gene. Genomic variances are denoted as changes in D1D2 rRNA as compared to the type strain control, ATCC® 18804™. The D1D2 region of eight strains was genetically identical, with a substitution from thymine to cytosine at position 455 as compared to the type strain, indicating they are closely related on a taxonomic level. Remaining strains exhibited either no change from the type strain, an insertional mutation, or a base pair substitution.

The ATCC® MP-8™ panel was also analyzed for resistance to seven antifungal drugs representing echinocandins, pyrimidine analogs, and triazoles. Each resistant strain showed resistance to one or more class of antifungal drugs. In particular, resistance to triazole antifungal drugs, Voriconazole, Itraconazole, and Fluconazole, was evident in 10 of the 12 resistant strains (depicted in the table). Resistance to echinocandins, including Anidulafungin, Micafungin, and Caspofungin, as well as resistance to the pyrimidine analog, 5-Flucytosine, was less common among strains. Resistance to these antifungal drugs was not dependent on the D1D2 genotype. Because these strains are taxonomically comparable and are unique in antifungal resistance, the ATCC® MP-8™ panel is ideal for use in drug discovery.

### Conclusion

The phenotypic and genotypic characterization of ATCC® MP-8™ strains illustrates that this set of *C. albicans* is well suited for testing novel antifungal therapies.

ATCC® No.	D1D2 Variance*	Anidulafungin	Micafungin	Caspofungin	5-Flucytosine	Voriconazole	Itraconazole	Fluconazole
<b>Resistant strains</b>								
64124™	T455C	R	R	R	I	R	R	R
10231™	T455C ^601A	R	S	S	S	R	R	R
76485™	T455C	R	S	R	S	S	S	S
28121™	C263T T4550 ^601TT	S	S	S	S	R	R	R
90819™	A570G ^601T	S	S	S	S	R	R	R
MYA-1023™	T455C A570G ^601A	S	S	S	S	R	R	R
MYA-427™	None	S	S	S	S	R	R	R
MYA-574™	T455C	S	S	S	S	R	R	R
38289™	T455C	S	S	S	S	R	R	S
11651™	T455C	S	S	S	S	S	R	S
96901™	T455C	S	S	S	S	S	S	R
90029™	T455C	S	S	S	R	S	S	S
<b>Sensitive Control Strains</b>								
MYA-2876™	T455C	S	S	S	S	S	S	S
18804™	Type Strain	S	S	S	S	S	S	S

The interpretation of resistance (R), intermediate (I), and susceptibility (S) was made according to guidelines described in the vendor's product instruction.

\*D1D2 variance refers to the nucleotide difference in the D1D2 region of LSU rDNA as compared to *Candida albicans* Type Strain (ATCC® 18804™). The nucleotide position is referenced using GenBank NW\_139715 (*C. albicans* SC5314 28S rRNA gene) annotation. A nucleotide substitution at position 455 from T (in the Type Strain) to C is expressed as T455C. The ^ symbol denotes an insertion at a given position, e.g., ^601TT denotes an insertion of two T's at position 601.

## References

1. Moudgal V, Sobel J. Antifungal s to treat *Candida albicans*. Expert Opin Pharmacother. 11(12): 2037-2048, 2010.
2. Huang M, Kao, KC. Population dynamics and the evolution of antifungal drug resistance in *Candida albicans*. FEMS Microbiol Lett (2012) 333(2):85-93.